Food Research

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INSIDE SCIENCE

The Vital Story of BREAKFAST CEREALS

with essential vitamins and minerals restored

by Science Writer

This article is one of a series devoted to the story of vitamin enriched or restored cereal products: white bread and rolls, white flour, corn meal and grits, macaroni products, white rice, breakfast cereals, farina.

Increasing in popularity over the years, breakfast cereals are used throughout the United States by young and old alike. Eaten with fruit and milk or light cream, they contribute an excellent combination of basic, flavorful, nutritious foods to the diet of children and adults.

In the past decade the use of these foods has grown markedly. In fact, it is estimated that there has been a 20% increase during the period. In 1953 the increase was much higher than the average of previous years. It was 5.5%.

During 1953, for example, many tons of breakfast cereal foods were eaten in the United States. Industry figures show that 4.6 pounds of dry cereals and 2.5 pounds of hot (cooked) cereals per person were consumed.

Many grains are processed to make breakfast cereals: wheat, corn, rice, oats. Often these foods may be eaten just as they come from the manufacturer's package. Or they may require only a short period of cooking to be ready for the table. They appeal to busy home-makers, as well as to institutional dietitians, because they are readily available in a variety of flavors to add interest and value to an important, but sometimes neglected, meal. They are used in cookery, too.

A Nutritional Advance

As the science of nutrition advanced in recent years, it was found that losses of essential vitamins and minerals occurred during processing of wheat, corn and rice into breakfast foods. When this became know, most manufacturers acted to overcome the losses. They adopted restoration.

Restoration simply means that certain vitamins and minerals are added to the cereal food during processing so that the finished product is generally equal to the whole grain values of those elements. The vitamins thiamine (B_1), riboflavin (B_2), and niacin as well as the mineral, iron, are used in restoration. Vitamin D is also added sometimes.

The recent trend toward pre-sweetened cold cereals for breakfast consumption and between-meal snacks emphasizes the nutritional importance of vitamin fortification. Increased calories require more "B" vitamins for proper utilization.

What the Vitamins Do

Thiamine is the vitamin which helps to build and maintain physical and mental health. It is essential for normal appetite, intestinal activity and sound nerves.

Niacin, which is another of the "B" vitamins, is needed for healthy body tissues. Its use in the American diet has done much to make a serious disease called pellagra disappear.

Riboflavin is the vitamin which is essential for growth. It helps to keep body tissues healthy and to maintain proper function of the eyes.

Vitamin D is important to prevent rickets in children and the development of certain abnormal bone conditions in adults.

Iron is essential for making good red blood and for preventing nutritional anemia.

Where Do The Vitamins Come From?

At about the same time that processing losses in breakfast cereals became known, other developments in the science of nutrition produced ample supplies of vitamins at economical prices. Thus, the quality of breakfast cereal foods could be, and was, greatly improved through restoration.

Since the early days of breakfast food restoration and of white flour and white bread enrichment, the world-famous firm of Hoffmann-La Roche has supplied top quality vitamins by the tons. Pioneering work in its laboratories and by its collaborators resulted in the "duplication" of some of Nature's extremely complex substances. First, the chemical composition of the vitamin was learned. Second, the pure substance was isolated. Third, the "duplicate" was made by synthesis. And fourth, the laboratory techniques were extended to large scale operations.

The manufactured "duplicate" is identical chemically and in biological activity with Nature's own product. A vitamin is still a vitamin regardless of its source. So efficient is large scale manufacturing that vitamins are sold at a lower cost than if they were extracted from natural sources.

Reprints of this article, and of all others in this series, are available without charge. Please send your request to the Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul Street, West; Montreal, Quebec.

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THE PECTOLYTIC ACTIVITY OF EXTRACTS OF DIFFERENT TYPES OF ONIONS[a,b]

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(Manuscript received July 24, 1954)

Different types of onions vary considerably in their resistance to decomposition by various molds. Since the determination of the relationship between certain pectolytic enzymes and pectic material has become an important tool for investigating plant tissue degeneration, the present study was undertaken to determine the polygalacturonase (PG) activity in onion extracts of various fungal contaminants known to infect onions. In addition, a study of the pectinesterase (PE) titer of different types of onions was made to determine whether or not a relationship between the enzyme and susceptibility to mold infection exists. The effect of PE on the general keeping quality of the types of onions studied was also observed.

LITERATURE REVIEW

There are numerous molds which infect onion bulbs. Weiss and O'Brien *(10)* have listed many including *Penicillium* sp., *Mucor* sp., *Aspergillus* sp., *Alternaria* sp., *Fusarium* sp., and *Botrytis cinerea*. These organisms are parasitic on both white and colored onions. Kertesz *(4)* has listed all of these organisms as producers of pectic enzymes.

But the literature is meager when dealing with the factors controlling mold growth on onions. Different degrees of resistance to mold growth are exhibited by different types of onions. Walker and Lindegreen *(8)* reported colored onion varieties to be not only highly resistant to *Collototrichum circinans* (a non-parasite on onions) but relatively free from infection by *Botrytis allii*, a normal onion pathogen. *Aspergillus niger*, however, attacked both colored and white bulbs. Walker *(7)* attempted to explain resistance by his observation that the epidermal cells of the fleshy scales of colored varieties of onions contain phenolic pigment compounds in the cell sap. However, he noted that the pigment was destroyed as soon as the mold penetrated the host cuticle and before the hyphae penetrated the cell lumen containing the phenolic compounds. Walker and Link *(9)* tested the effect of 21 phenolic compounds in standard broth against the growth of organisms, including the three studied by Walker and Lindegreen *(8)*. They found that *Aspergillus niger* was most resistant to the influence of the phenolic compounds tested; *Botrytis allii* and *Collototrichum circinans* followed in decreasing order of resistance. It has been generally observed by those working with onions that the white are more susceptible to infection than the colored types and in addition are the poorest storage varieties. Conversely, the yellow and the brown types are the most resistant to infection and the best keepers. Bermuda types and the red types appear to be somewhat intermediate in their properties of resistance to infection and storage effects.

EXPERIMENTAL

Polygalacturonase-like activity produced by molds in onion extract. *Cultures used.* Cultures identified as *Fusarium oxysporum*, *Botrytis cinerea*, *Alternaria humicola*, *Aspergillus niger*, *Penicillium* sp., and *Mucor* sp. were grown for 4 weeks on potato-dextrose

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[a] Journal article No. 1640.

[b] Presented at the Fourteenth Annual Meeting of IFT, Los Angeles, June 29, 1954.

agar slants (Difco). Spore suspensions of these cultures were then inoculated into extracts of 3 types of onions—Bermuda, yellow, and white.

Extraction of onions. Sound whole onions were peeled and macerated in a Waring blender. The macerate was then transferred to several layers of cheesecloth and the cell sap was squeezed out by hand. Twenty ml. of the cell sap was then placed into each of a series of 125 ml. Erlenmeyer flasks. Five flasks of extract from each type of onion were prepared for each culture studied. The flasks were plugged with cotton, sterilized by autoclaving, and stored at refrigeration temperature until used. Alcohol precipitation tests indicated that no appreciable amount of pectin was present in the extracts.

Inoculation. A spore suspension of each mold culture was prepared and standardized to contain 500 spores per ml. by use of a haemocytometer counting chamber. One ml. of this suspension was inoculated into each of a series of flasks. Four flasks of sterile extract of each type of onion were used for each mold. The cultures were incubated at 30° ± 1°C.

Polygalacturonase-like enzyme activity determination. The method used in the determination of the PG-like enzyme activity was a modification of that of Bell, Etchells, and Jones *(1)*. Due to the high viscosity of the pectin[e] suspension employed, a 1.5% suspension was used in place of the 3% suspension.

At 1.5, 2.5, 3.5, and 4.5 days incubation one flask was removed from incubation for each mold and type of onion. The cell sap was observed for the presence of mat formation, layered with toluene, and the pH was measured. Two 5-ml. portions were withdrawn from each flask. One portion was heated to 80°C. for 10 minutes in a water bath to serve as the control, and the other was inoculated into 25 ml. of pectin in phthalate buffer at pH 4.0. The control was also inoculated into the buffered pectin solution. These samples were then incubated for 7 days at 30° ± 1°C. At the end of the incubation period, the dropping times through a 25-ml. pipette were recorded, and the per cent loss of viscosity was determined.

RESULTS

The presence of polygalacturonase-like enzymes. Results of the study of the production of the PG-like enzymes are presented in Figures 1, 2, and 3. In addition data are presented on the pH changes occurring in the extracts. There appeared to be a relationship between the appearance of visible growth and a change in pH. A change in the pH of the extract was noted either at the same time as the appearance of visible growth, or followed the growth by one day. Therefore, the first pH changes noted in Figures 1, 2, and 3 were an indication of the presence of macroscopic growth.

Figure 1 shows the relationship of pH changes, and the change in the PG-like enzyme activity produced by *Botrytis cinerea* and *Fusarium oxysporum* when grown in the extracts of 3 types of onions. *Botrytis cinerea* produced no change in pH throughout the duration of the experiment except when growing on the Bermuda extract wherein a slight decrease was noted at 3.5 and 4.5 days. With all 3 extracts PG-like activity was noted to be similar and sufficient enzyme was produced on each extract to cause a loss of viscosity in the maximum range measurable by this method.

Changes in pH produced by *Fusarium oxysporum* were similar in all 3 extracts. However, the *Fusarium* mold did not produce PG-like enzyme activity rapidly. The onion extracts resulted in no appreciable loss of viscosity of the pectin suspension until after 3.5 days of incubation. Only with the white onion extract was a maximum reduction in the viscosity of the pectin suspension noted after 4.5 days of growth of this mold.

Figure 2 demonstrates the relationship of pH change and change in the per cent loss of viscosity of a standard pectin suspension over a period of time produced by *Alternaria humicola* and *Penicillium* sp. It can be seen that the pH changes produced by *Alternaria humicola* were quite similar in the 3 extracts. No change was noted during the first 2.5 days. At this time there was a slight increase of pH followed by a decrease. The PG-like activity resulting from the growth of *Alternaria humicola* was similar in all

[e] Pectin No. D-890 supplies by Sunkist Growers, Research Department, Corona, California.

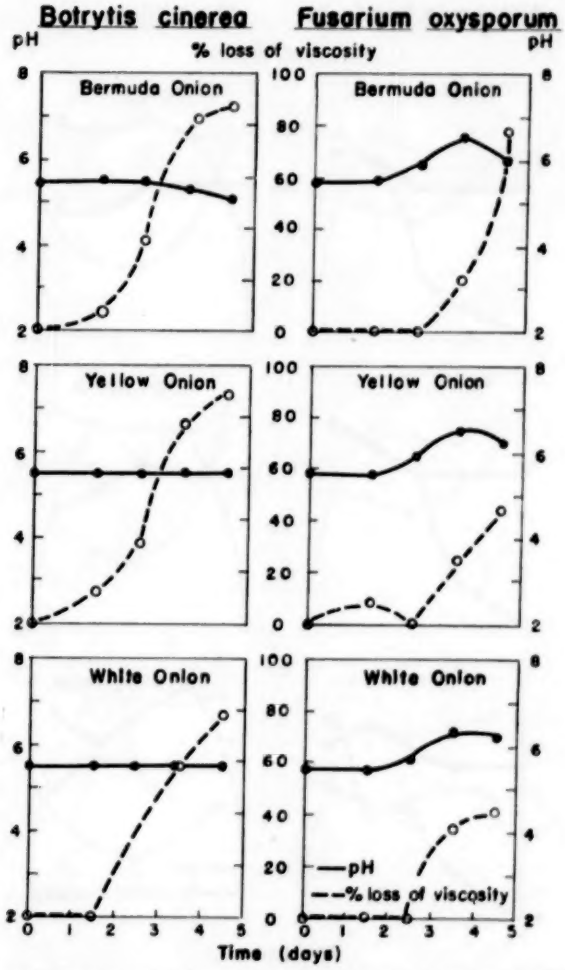

Figure 1. pH changes and elaboration of PG-like enzyme expressed in units of per cent loss of viscosity of a standard pectin suspension by two molds on three onion extracts.

3 extracts. At the end of 4.5 days of incubation of the inoculated extracts, the PG-like activity of all 3 extracts was sufficient to reduce the viscosity of the standard pectin suspension by 70-80%.

Growth of *Penicillium* sp. resulted in pH changes in the 3 extracts similar to those produced by *Alternatia humicola* and *Fusarium oxysporum*. There appeared to be a slight increase in pH at 2.5 days on the yellow extract, while the initial change in pH on the other extracts occurred at 3.5 days. The significance of this variation is doubted since the initial obvious mat formation occurred at 2.5 days in all cases wherein *Penicillium* sp. was involved.

Penicillium sp. differed from the other 3 organisms discussed so far in its ability to produce PG-like enzyme very rapidly on all 3 extracts. This mold produced sufficient

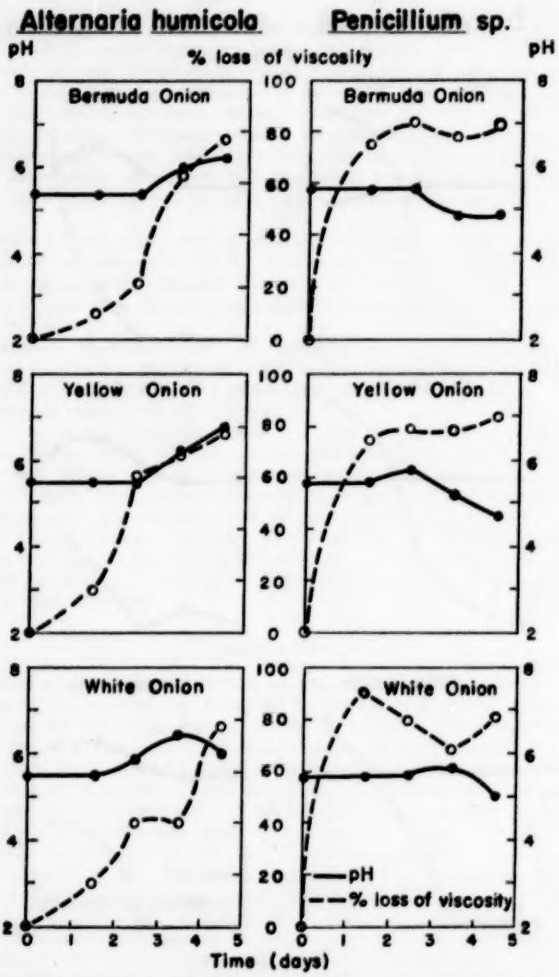

Figure 2. pH changes and elaboration of PG-like enzyme expressed in units of per cent loss of viscosity of a standard pectin suspension by two molds on three onion extracts.

enzyme to cause a maximum per cent loss of viscosity at the end of 1.5 days. After 2.5-3.5 days growth of *Penicillium* sp. a decrease in the loss of viscosity from 80 to approximately 70% was noted. However, this was not believed to be significant.

Figure 3 shows the relationship of pH change and production of PG-like enzymes for *Aspergillus niger* and *Mucor* sp. grown on the 3 substrates. *Aspergillus niger* produced a rapid change in the pH of all 3 extracts which corresponded to rapid mat formation. The general trend of pH change was similar for all 3 extracts. However, the pH of the Bermuda and yellow onion extracts were in the range 6.6-7.2 at the end of the experiment, while the final pH of the white onion extract was 4.8. However, after 5.5 days of incubation the pH of the white onion extract was found to be about equal to that of the other two.

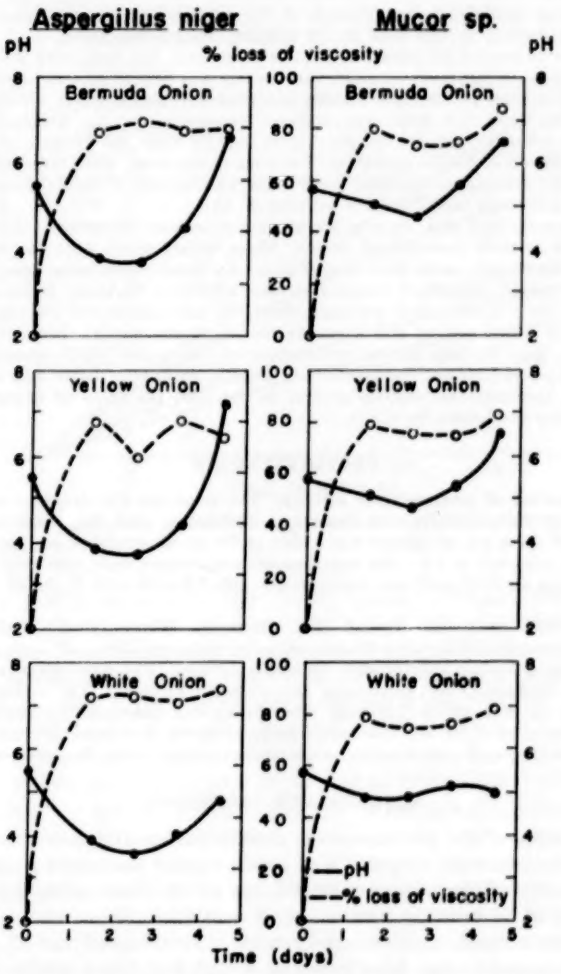

Figure 3. pH changes and elaboration of PG-like enzyme expressed in units of per cent loss of viscosity of a standard pectin suspension by two molds on three onion extracts.

The production of PG-like enzymes in all 3 substrates was rapid. The extracts in which *Aspergillus niger* was grown caused per cent loss of viscosity in the maximum range at the end of 1.5 days. The activity of the PG-like enzymes remained high throughout the duration of the experiment.

Mucor sp. caused similar pH changes in Bermuda and yellow onion extracts. However, the pH of the white onion extract did not increase as rapidly after 3.5-4.5 days of incubation as did the pH of the other 2 extracts. The significance of this is doubtful, since mat formation was similar on all 3 extracts.

As in the cases of *Penicillium* sp. and *Aspergillus niger*, *Mucor* sp. produced a maximum measurable titer of PG-like enzyme at the end of 1.5 days, and this titer

was maintained throughout the duration of the experiment. No significant difference in enzyme production by this mold in the 3 onion extracts was noted.

The 6 molds studied all produced PG-like enzyme(s) but they were divided into 2 groups according to the speed at which this enzyme was produced. *Alternaria humicola*, *Fusarium oxysporium* and *Botrytis cinerea* produced the enzyme slowly while *Penicillium* sp., *Aspergillus niger*, and *Mucor* sp. produced the enzyme rapidly. Although the latter groups were noted, in general, to grow more rapidly than the former, there was no obvious correlation of visible growth with enzyme production. With three of the molds, PG-like enzyme activity was evident prior to the appearance of visible growth. Similar observations have been made by other workers *(5, 11)*.

It is generally held that there is a relationship between intensity of coloration and the resistance to mold infection of onions. Those onions which were the least colored (white and Bermuda), were more susceptible, and those which were more pigmented (yellow and brown) were more resistant to mold infection. However, it was shown here that each of the 6 molds tested produced about the same amount of PG-like enzyme in the extract of white, yellow, and Bermuda onions. Consequently, there must be some factor other than PG-like enzyme production of the molds which accounts for the resistance of colored onions. To ascertain if possible what this factor was, a study was made of the pectinesterase enzyme present in the different types of onions and their ability to resist breakdown by molds.

PECTINESTERASE

Determination of pectinesterase activity. The substrate was prepared according to the method of Bell, Etchells, and Jones *(2)* modified so that the substrate contained 1.0% pectin.[4] Five ml. of extract was added to 50 ml. of substrate and the pH of the mixture was adjusted to 7.5. The enzyme-substrate mixture was permitted to incubate for 30 minutes at 30°C. and was readjusted to pH 7.5 with 0.01 N NaOH at 5-minute intervals.

Studies were made on Crystal Wax, Bermuda, White (Utah), Sweet Spanish (Texas), Yellow, and Australian Brown onions by weighing 500 g. of each type, macerating them in a Waring blender with 2% NaCl solution. The macerate was then transferred to 8 thicknesses of cheese-cloth and squeezed by hand. The extract was sufficiently clear so that filtering through filter paper was unnecessary. The extract was then transferred to a 500 ml. volumetric flask, made up to volume by the addition of distilled water, layered with toluene, and stored at refrigeration temperature until used.

RESULTS AND DISCUSSION

The results of the pectinesterase determination are shown in Figure 4. It can be seen that the Crystal Wax onion extract contained a significantly higher activity of the enzyme than did any of the other onion types studied. At the end of 30 minutes incubation, it consumed, in comparison with the yellow onion extract, approximately twice the volume of NaOH. This particular phenomenon has been noted at 4 different times under 4 different sets of experimental conditions in reactions carried to completion. Figure 4 also shows that at the end of the determination, the activity of the Crystal Wax onion extract was a straight line function, indicating a high activity of the enzyme in comparison to the other extracts which were not straight line functions during any portion of the experiment.

Yellow and Australian Brown onion extracts showed the lowest activity of PE, the activity of the Australian Brown being slightly less than that of the yellow; this may or may not be significant.

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[4] Pectin No. 444-B-6 supplied by Sunkist Growers, Inc., Research Department, Corona, California.

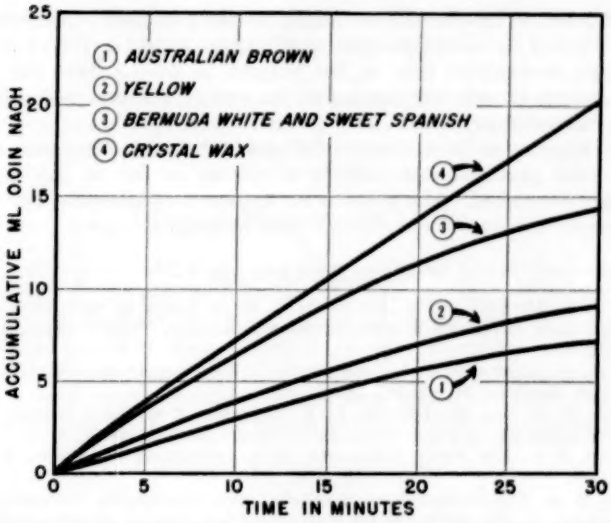

Figure 4. Graph showing the amount of pectinesterase present in extracts of different types of onions.

Figure 4 also shows that the Sweet Spanish (Texas) and Bermuda White (Utah) onions had the same PE activity in this experiment, an activity mid-way between that of Crystal Wax and Yellow. It was felt that this particular Sweet Spanish onion may have been a cross with some other type(s), since it had the typical markings of the Spanish on its outer scale. But it was much smaller than the typical Sweet Spanish onions previously studied. Previous experiments with Sweet Spanish onions indicated that it had an enzyme activity similar to that of the Yellow variety.

This study is not definitive, since the onion samples employed were store-purchased and later identified. However, the difference in activity between the yellow and white onion types has been observed often enough to become significant, and the activity of the other onion types tends to confirm the relationship of PE activity to the resistance of onions to mold infection. It also suggests that the amount of PE present may be a good index to keeping quality of onions during storage.

SUMMARY

Six mold cultures showed growth and PG-like enzyme production in varying degrees on three types of onion extracts. Each culture behaved similarly in regard to pH change and enzyme production on all three types of onion extracts. The molds could be placed into two main groups according to their ability to produce PG-like enzyme: (a) those cultures which produced the enzyme rapidly, *viz. Penicillium* sp., *Aspergillus niger*, and *Mucor* sp.; and (b) those cultures which produced the enzyme slowly, *viz. Alternaria humicola, Fusarium oxysporum*, and *Botrytis cinerea*.

Penicillium sp. produced a maximum measurable titer of enzyme in 1.5 days but showed no visible growth for 2.5 days. *Botrytis cinerea* produced a maximum measurable titer of the enzyme in 2.5-3.5 days but showed signs of growth in only one extract at the end of 4.5 days indicating that enzyme production preceded visible growth.

There appears to be a direct relationship between resistance to mold infection and pectinesterase content in onions so far as the organisms studied are concerned. Also there is an apparent relationship between the pectinesterase content of onions and their keeping quality.

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A SIMPLIFIED MEDIUM FOR COUNTING *CLOSTRIDIUM* SPORES[a,b]

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(Manuscript received August 7, 1954)

In a comparative study of media for counting *Clostridium* spores, it was reported by Wynne and Foster *(5)* that a modified Yesair's pork infusion agar with added soluble starch yielded the highest counts. Although this medium has been used routinely in our laboratory and elsewhere *(2, 4)*, it is expensive and difficult to prepare. The present investigation concerns a yeast extract medium offered as a possible replacement for the pork infusion and other complex media currently used for counting spores.

MATERIALS AND METHODS

Test strains[d] used were *Cl. botulinum* (62A and 115B), *Cl. feseri*, *Cl. histolyticum*, *Cl. perfringens* and Putrefactive Anaerobe No. 3679. Spore suspensions were prepared from 14-day cultures in Difco brain heart infusion broth containing 0.25% BBL thioglycollate supplement. Cultures were heated 20 minutes at 75°C. and washed 4 times. The spores were then suspended in sterile distilled water containing glass beads, reheated for 20 minutes at 75°C., and agitated one hour on a Kahn shaker.

The basal yeast extract medium contained, per liter: Difco yeast extract, 10 g.; BBL thioglycollate supplement, 5 g.; soluble starch, 1 g.; K_2HPO_4, 2 g.; and agar, 15 g. The pH was adjusted to approximately 7.4 with NaOH prior to addition of the agar. Separate sterilization of the K_2HPO_4 improved the clarity of the medium.

The yeast extract starch medium was compared with Yesair's pork infusion medium as modified by Wynne and Foster *(5)* to contain BBL thioglycollate supplement and 0.1% soluble starch. Both media were tubed in approximately 15-ml. amounts in Prickett (Fisher modified agar slant) tubes. In view of the recent report by Andersen *(1)* that addition of $NaHCO_3$ to infusion media resulted in higher counts and earlier appearance of colonies, the effect of this compound was evaluated with both media. When bicarbonate was used, 0.3 ml. of a Seitz-filtered 5% solution was added per tube just prior to inoculation. After solidification of all inoculated media, to each tube was added an overlay of 3-4 ml. of 1.5% agar containing 0.5% BBL thioglycollate supplement.

Mildly heated spores. Suspensions diluted to contain approximately 50-100 spores per ml. were heated for 20 minutes at 75°C. to remove dissolved oxygen and effect possible heat activation *(3)*. Since the spores had been heated 40 minutes at 75°C. during preparation of the suspensions, their total heat treatment was 60 minutes at 75°C. One ml. of heated suspension was pipetted into each of 9 replicate tubes of each medium. The overall counting error was estimated at ±10%. Incubation was for 24-72 hours, with check counts after 4 weeks.

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[b] This investigation was supported in part by research grant C-1507 from the National Cancer Institute of the National Institutes of Health, Public Health Service, and an institutional grant from the American Cancer Society.

[c] Present address: State Department of Health Laboratories, Oklahoma City, Oklahoma.

[d] All obtained from the Department of Bacteriology, The University of Texas, Austin, Texas.

Drastically heated spores. Suspensions were diluted to contain approximately 25,000 to 50,000 spores per ml. Following exposure to 75°C. for 5 minutes to effect any possible heat activation, platings were made for initial spore levels. The spores were then heated in a boiling water bath for a period sufficient to secure at least 99.95% lethality. Both initial and recovery spore counts were made from duplicate counts from each of triplicate tubes. Incubation was for 72 hours, with check counts after 3 months.

RESULTS AND DISCUSSION

Mildly heated spores. With all strains tested, counts in yeast extract starch bicarbonate (YESB) agar were comparable to those obtained in modified Yesair's or in the Yesair medium plus bicarbonate (Table 1). This finding was repeatedly confirmed. Check counts after 4 weeks' incubation showed no increases.

In agreement with the results obtained in a recent spore counting survey sponsored by the Western Regional Research Laboratory,[e] addition of bicarbonate to modified Yesair's medium resulted in earlier appearance of colonies with *Cl. botulinum* 62A. Maximum counts were obtained in the bicarbonate-supplemented medium after an incubation period as short as 24 hours, while a period of at least 48 hours was necessary when bicarbonate was omitted. Furthermore, addition of bicarbonate to this medium resulted in a 47% increase in the mean count with *Cl. botulinum* 62A (Table 1). Statistical analysis by the "t" test indicated this increment to be highly significant ($P < 0.01$); two separate experiments showed increases in counts due to added bicarbonate of 70% and 100%, respectively. With the yeast extract starch medium, added bicarbonate consistently resulted in a small but statistically significant increase in counts with *Cl. botulinum* 115B (Table 1). At times increased counts were also observed with *Cl. feseri* and *Cl. perfringens.*

Drastically heated spores. Mean recovery counts with YESB agar and modified Yesair's agar were not significantly different (Table 1). Check counts after 3 months showed no evidence of dormancy. Supplementation of the Yesair medium with bicarbonate had no demonstrable effect on counts of drastically heated spores of *Cl. botulinum* 62A.

These findings, though admittedly limited in scope, suggest further investigation of YESB medium as a recovery medium for drastically heated *Clostridium* spores.

SUMMARY

A simple, easily prepared medium for counting *Clostridium* spores is described. With yeast extract starch bicarbonate (YESB) agar, counts of mildly heated (75°C. for 60 minutes) spores of 5 *Clostridium* strains were consistently comparable to those secured in a modification of Yesair's pork infusion medium, with or without added bicarbonate. Omission of bicarbonate from YESB agar resulted consistently in a small but significant decrease in counts with *Cl. botulinum* 115B. Addition of bicarbonate to the Yesair medium resulted in increased counts and earlier colony development with *Cl. botulinum* 62A.

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[e] Copies of the report of this spore counting project are available from Dr. H. C. Olcott, Western Regional Research Laboratory, Albany 6, California.

TABLE 1

Comparative means counts of Clostridium spores

Organism	Mildly heated spores (75°C. for 60 min.)				Drastically heated spores (100°C.)		
	Modified Yesair's	Modified Yesair's with $NaHCO_3$	Yeast extract starch	Yeast extract starch bicarbonate	Heating time (min.)	Modified Yesair's[1]	Yeast extract starch bicarbonate[1]
Cl. botulinum 62A	81	119	....	123	0	56,000	57,000
					60	12 (99.98)	11 (99.98)
Cl. botulinum 115B	57	62	46	62	0	43,000	36,000
					30	7 (99.98)	9 (99.98)
Cl. feseri	46	47	36	48	0	56,000	48,000
					5	4 (99.99)	6 (99.99)
Cl. histolyticum	....	....	....	....	0	24,000	27,000
					10	8 (99.97)	8 (99.97)
Cl. perfringens	41	49	44	55	0	48,000	38,000
					5	5 (99.99)	6 (99.98)
P.A. 3679	44	37	43	45	0	45,000	45,000
					10	11 (99.98)	10 (99.98)

[1] Figures in parentheses indicate % destruction.

With spores of 6 *Clostridium* strains heated at 100°C. for a period sufficient to insure at least 99.95% destruction, recovery counts in YESB were comparable to those in modified Yesair's medium, with no evidence of dormancy.

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STUDIES OF CERTAIN RED PIGMENTS FORMED FROM 2-THIOBARBITURIC ACID[a]

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The reaction between thiobarbituric acid and lipid oxidation products that yields a red color has been used as a measure of oxidative deterioration in foods. The chemistry of the reaction is largely unknown, but several workers have noted the correlation between the intensity of the color and the degree of fat oxidation. The present studies were directed toward characterization and identification of the red pigment(s), in the hope that the results would yield information on the compounds responsible for oxidative flavor defects. Although the results obtained are largely negative, they indicate that the reactions involving the red pigment may be more complex than has been previously assumed. The characteristics of the red pigment and the nature of its degradation products limit conventional approaches toward production of a pure material. An indirect method of determining the proportionate composition of the pigment is presented. The formation of a second red pigment from the same reactants has been demonstrated.

Bernheim, Bernheim and Wilbur *(1)* established that lipid oxidation products reacted with thiobarbituric acid to produce a characteristic red color. Subsequent investigations have indicated that the reaction can serve as a measure of oxidation in edible fats and oils *(2, 6, 7, 11, 12, 16, 17)*.

Reactions of various aldehydes with barbituric acid *(3, 4, 18)* and thiobarbituric acid *(4, 5, 13)* have been reported. In most of the studies it was assumed that a simple condensation reaction was involved; proposed structures were based principally on combustion analyses.

Kohn and Liversedge *(10)* showed that tissue suspensions or slices incubated aerobically produced a compound that will condense with either p-aminobenzoic acid or thiobarbituric acid, respectively producing yellow or orange-red colors. Bernheim *et al. (1)* concluded that the colors obtained by Kohn and Liversedge were due to an oxidation product of unsaturated fatty acids, particularly linolenic acid. They reported isolation of the thiobarbituric acid pigment, which contained some inorganic residue but no organic phosphate. On the basis of a combustion analysis, corrected for inorganic residue, it was suggested that a three carbon chain containing one oxygen atom may have added to the thiobarbituric acid molecule.

Patton and Kurtz *(12)* established that a pigment produced by thiobarbituric acid and malonaldehyde had the same visible absorption characteristics as that produced by thiobarbituric acid and oxidized milk fat.

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Kurtz, Price, and Patton *(11)* tentatively identified the compound in oxidized milk fat that is responsible for the results of the thiobarbituric acid test as malonaldehyde and postulated that the reaction occurred by attack of the monoenolic form of malonaldehyde on the active methylene group of thiobarbituric acid, followed by ring closure.

Kohn *(9)* reported that thiobarbituric acid was a specific reagent for sulfadiazine among the sulfonamides, and suggested that the reaction involved the amino pyrimidine moiety. Later, Shepherd *(13)* established that the color-forming reaction was specific for the pyrimidine ring. Among the transmission curves presented was one for a 2-sulfanilamidopyrimidine-thiobarbituric acid pigment. It was recognized that the chemistry of the reaction and the nature of the reaction product remained to be elucidated.

EXPERIMENTAL

Preparation of pigments. *Oxidized milk fat-thiobarbituric acid pigment.* Milk fat was placed in 500 ml. Erlenmeyer flasks and exposed to sunlight for a period of several months. Under such treatment the color disappeared and the fat exhibited an intensely tallowy odor and flavor. This material was stored under refrigeration.

One g. of 2-thiobarbituric acid was dissolved in 2 l. of distilled water in a 5-l. round bottom flask. Approximately 500 g. of oxidized milk fat was added to the flask and the mixture refluxed with agitation for a period of 3-4 hours. The two phases were allowed to separate, the flask was chilled, and the solidified fat was removed. To react the thiobarbituric acid more completely, additional portions of oxidized fat were added to the flask and the above procedure repeated until a colorimeter failed to detect further increase in color production during a 30-min. period. This usually required 6 to 7 portions of oxidized fat. The chilled aqueous solution was filtered, and extracted with ethyl ether. Evaporation to dryness yielded a mixture of unreacted thiobarbituric acid and red pigment.

Malonaldehyde-thiobarbituric acid pigment. Dilute hydrochloric acid was boiled in a 3-necked round bottom flask fitted with a Friedrichs condenser and separatory funnel. Approximately 50 g. of $\Delta^{1:2\text{-}3}$, 3-dimethoxysodiumpropoxide *(19)* dissolved in distilled water was admitted, drop-wise, to the round bottom flask. The condensate, containing malonaldehyde, was delivered to a beaker containing a heated aqueous solution of thiobarbituric acid. After a period of 24-36 hours the contents of the beaker were concentrated by evaporation and cooled. The dark red to violet-purple crystals that formed were collected by filtration and recrystallized from boiling water. This crude pigment preparation probably contained some unreacted thiobarbituric acid.

2-Sulfanilamidopyrimidine-thiobarbituric acid pigment. Approximately 9 g. of 2-sulfanilamidopyrimidine dissolved in 6 l. of boiling distilled water was added to 16 g. of 2-thiobarbituric acid dissolved in 2 l. of boiling distilled water, and the mixture was heated for 2-3 hours on a steam bath. The yellowish-white crystalline precipitate that developed was removed by filtration. The crude red pigment was collected by evaporating the filtrate to dryness.

Pigment purification. Attempts to purify pigment preparations are described below. Subsequent work reported later herein indicates that techniques involving solution of the pigment generate impurities. The minute amount of pigment that does dissolve undergoes hydrolysis. One of the hydrolysis products is probably malonaldehyde, which is highly reactive and whose isolation has apparently never been achieved. Pigment preparations which had been subjected to a procedure involving solution would, on recovery, contain variable amounts of thiobarbituric acid and malonaldehyde or its reaction products.

Miscellaneous observations on the oxidized milk fat-thiobarbituric acid pigment. Studies of the red pigment preparations produced by the reaction of thiobarbituric acid and oxidized milk fat revealed certain general properties. Ultraviolet spectral analysis, discussed below, indicated that the pigment was contaminated with unreacted thiobarbituric acid. The material appeared to be heat stable up to approximately 260°C.,

where it decomposed. The addition of alkalis resulted in shifting the maximum to a longer wave length. The color compound is so sparingly soluble in water that no information concerning its base combining properties was obtained by titration.

Aqueous pigment solutions will readily decolorize iodine or bromine. The maximum at 528-532 mμ undergoes a decrease roughly proportionate to the amount of iodine added. Excess iodine eliminated the 528-532 mμ maximum, leaving a flattened maximum in the region 500-510 mμ. This latter may be due to a component responsible for the shoulder the normal curve exhibits in this vicinity.

Application of the thiobarbituric acid reaction to vegetable oils. Cottonseed oil, peanut oil, and linseed oil were oxidized by treatment with heat, ultraviolet radiation, or ethanolic cupric nitrate. The results of a qualitative thiobarbituric acid test applied to these oils indicated that thiobarbituric acid produces the same red color with oxidized vegetable oils as with oxidized milk fat.

Chromatographic adsorption. The pigments adsorbed strongly to precipitated milk proteins, filter paper, and a number of other materials which suggests that chromatographic adsorption might offer a means of isolation and purification. Solutions of the pigments in water, methyl alcohol, ethyl alcohol, acetone, n-butyl alcohol, and isoamyl alcohol were chromatographed on columns of charcoal, Celite, magnesium oxide, and

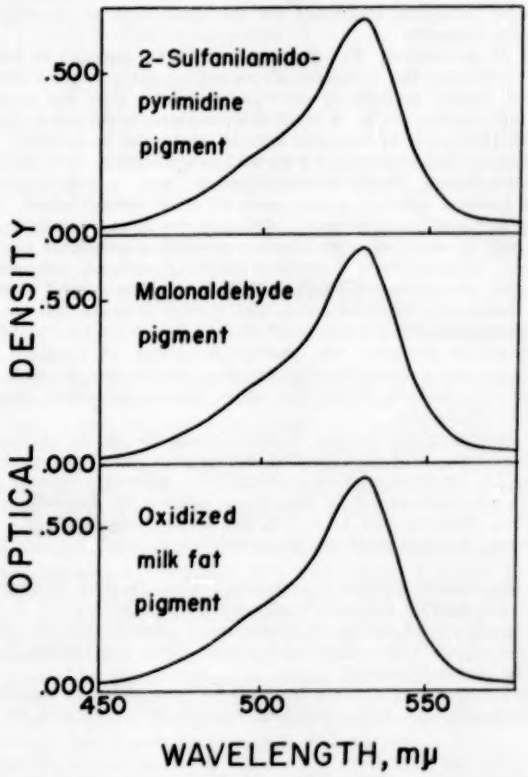

Figure 1. Visible spectra of pigments derived from 2-thiobarbituric acid and oxidized milk fat, malonaldehyde, and 2-sulfanilamidopyrimidine.

Florisil.[4] The less polar solvents (n-butyl alcohol and isoamyl alcohol) were also used on columns of sugar, starch, silicic acid, and clay. The only satisfactory results were obtained with Celite-Florisil columns developed with water. Concentration of eluates obtained by this procedure invariably yielded a white crystalline precipitate. This appeared to be a silicate from the column even though the adsorbant was given a preliminary washing. The amounts of pigment obtained by this procedure were so small that attempts to obtain pure fractions were unsuccessful.

On one occasion, development of a Celite-Florisil column with pH 9 buffer resulted in a relatively sharp separation of two red fractions. These fractions possessed maxima at 515 and 540 mμ. Attempts to repeat this fractionation were unsuccessful.

Various anion exchange resins (Amberlites*—IR-4AC, IR 4 B, and IR 400; Duolite† A-2) were also used in attempts to separate unreacted thiobarbituric acid from the pigments. All resins investigated adsorbed the color tenaciously. When the columns were washed with 20% HCl, thiobarbituric acid was present in the eluate. In an effort to determine whether this elution was quantitative, known additions of thiobarbituric acid were made to aliquots of aqueous solution, and the eluates recovered were analyzed spectrophotometrically. Plots of thiobarbituric acid recovered against added thiobarbituric acid were not linear. Subsequent work, reported below, indicates that hydrolysis of the pigments probably occurred under these conditions.

Solvent purification of pigment preparations. Various solvents and solvent combinations were used in attempts to remove impurities from the crude pigment preparations. Neither the use of extraction apparatus nor the application of recrystallization techniques yielded pure pigments.

Preparation of derivatives. The pigments would be expected to contain certain reactive groups. Although the formation of derivatives yields positive information concerning structure, failure to form a particular derivative does not necessarily imply the absence of that reactive group. A large bulky molecule is subject to steric hindrance and other factors that result in decreased activity might also be present.

Standard chemical procedures *(14)* were used in attempts to form derivatives of the pigments. Semi-carbazide, p-nitrophenylhydrazine, and 2, 4-dinitrophenylhydrazine, which normally detect a carbonyl group, were all tried without effect. Permanganate oxidation to an acid was also attempted. Although the permanganate was reduced, no acid was recovered by standard crystallization procedures and ether extraction of the reaction mixture. Hydroxyl-type derivatives—diphenylurethane, benzoate, p-nitrobenzoate, acetate, and nitrophthalate—were not obtained. Unsuccessful attempts to form the acetamide, benzamide, phenylthiourea, and picrate indicate that an amino group was not readily available for reaction.

Spectrophotometric analyses. The absorption spectra of pigments formed from thiobarbituric acid and 2-sulfanilamidopyrimidine, malonaldehyde, and oxidized milk fat, determined on a Beckman Model DU quartz spectrophotometer, were identical in the visible region (Figure 1).

Ultraviolet spectra proved helpful in establishing the purity of pigment preparations. 2-Thiobarbituric acid and 2-sulfanilamidopyrimidine have similar ultraviolet spectra (Figure 2). In water solution, both exhibit a primary maximum at 262-265 mμ, and a secondary maximum at 240-242 mμ. These maxima are evidently a characteristic of the pyrimidine structure. In 1.5 *N* HCl, the primary maximum of 2-thiobarbituric acid is at 282 mμ. Aqueous solutions of malonaldehyde exhibit a single maximum at 240 mμ.

In one of the chromatographic experiments a trace fraction of pigment from oxidized milk fat was isolated that exhibited essentially no absorption in the ultraviolet region. In using ultraviolet spectra as a measure of impurities in the pigments it was assumed that the pigments from malonaldehyde and from 2-sulfanilamidopyrimidine do not absorb in the ultraviolet region.

When known amounts of 2-thiobarbituric acid were added to aqueous solutions of the malonaldehyde pigment, both ultraviolet maxima were increased but there was no

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[4] A synthetic magnesium silicate, obtainable from the Floridin Company, Warren, Pa.

* Obtainable from Rohm and Haas Co., Philadelphia 5, Pa.

† Obtainable from Chemical Process Co., San Francisco 2, Calif.

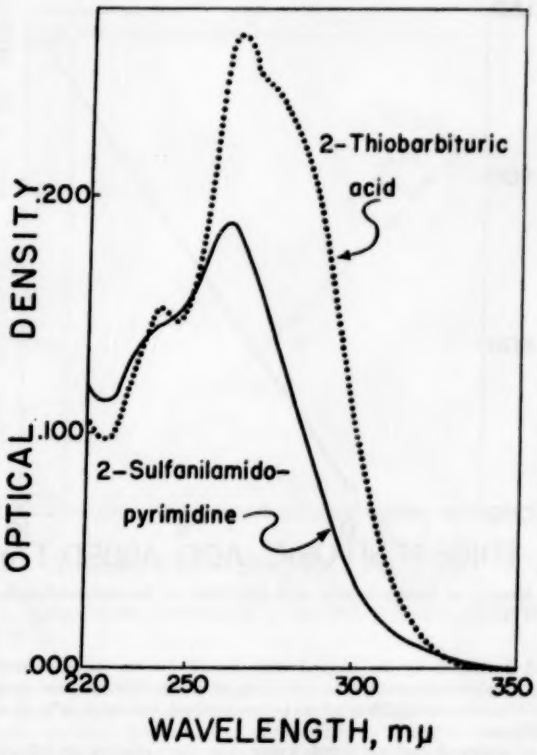

Figure 2. Ultraviolet spectra of 2-thiobarbituric acid and 2-sulfanilamidopyrimidine.

change in the visible maximum. When the concentration of thiobarbituric acid added was plotted against the primary ultraviolet maximum, the secondary ultraviolet maximum, or their sum, a general trend was evident. In 1.5 *N* HCl the relationship was linear, which was not the case in the region pH 2 to pH 7. Figure 3 illustrates a plot of the primary ultraviolet maximum against the concentration of added thiobarbituric acid. Assuming that the pure color compound does not absorb in the ultraviolet, extrapolation to zero optical density would give the concentration of unreacted thiobarbituric acid originally present in the sample.

Degradation of malonaldehyde color. Subjecting solutions of the malonaldehyde-thiobarbituric acid pigment in 1.5 *N* HCl to a temperature of 100°C. for 24-36 hours resulted in disappearance of the color and in increase in the optical density at 235 mμ. When aliquots of color solution containing known additions of thiobarbituric acid were treated in this manner the optical density at 235 mμ was a straight line function of the amount of thiobarbituric acid added (Figure 4). Both malonaldehyde and thiobarbituric acid disappear under the conditions employed. The rate of disappearance of the former is fairly rapid, and the degradation products do not absorb in the region 220-600 mμ. In contrast, thiobarbituric acid gives rise to a material that exhibits an absorption maximum at 235 mμ, which disappears in turn at a slower rate.

Assuming that the optical density at 235 mμ is a measure of the total concentration of thiobarbituric acid, extrapolation to zero optical density would give the total concentration of thiobarbituric acid obtained by degradation of the original color sample. The difference between the corrected weight of the sample and the weight of the thio-

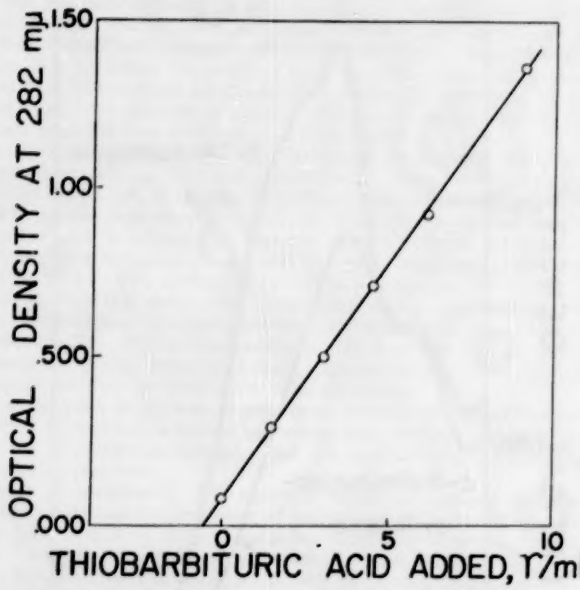

Figure 3. Results of thiobarbituric acid additions to the malonaldehyde derived pigment in 1.5 N HCl.

barbituric acid produced by its degradation should be due to malonaldehyde. Data treated in this manner indicated that 1.0 mole of thiobarbituric acid reacted with 1.0 mole of malonaldehyde without loss of water to produce the color with absorption maximum at 528-532 mμ.

Isolation of a second malonaldehyde color. On one occasion, an effort was made to exhaust the unreacted thiobarbituric acid by distilling malonaldehyde into a heated solution of thiobarbituric acid for a period of several days. The color product that was isolated by evaporation to dryness exhibited its maximum at 507 mμ (Figure 5). The maximum shifted to the usual 528–532 mμ position when a solution of this material was heated in the presence of added thiobarbituric acid. Spectral examination of the pigment with maximum at 507 mμ before and after degradation, by the procedure described above, indicated that three molecules of malonaldehyde had reacted with one of thiobarbituric acid.

Ratio of occurrence of free thiobarbituric acid and free malonaldehyde in color preparations. No preparations were obtained whose spectra revealed them to be completely free of unreacted thiobarbituric acid and malonaldehyde. The pigments were usually dissolved by boiling 8-10 mg. for 5-10 minutes in 500 cc. of distilled water. The ultraviolet maxima characteristic of the parent compounds increased, and the maximum at 528-532 mμ decreased during boiling and subsequent holding of the solution. The ultraviolet maxima of these solutions of recrystallized color were probably due to thiobarbituric acid and malonaldehyde freed by hydrolysis of part of the pigment. If this were so, then their relative concentrations should be constant, and should indicate their combining proportion.

Solutions containing malonaldehyde were prepared by collecting the distillate from the acid hydrolysis at 100°C. of $\Delta^{1:2}$-3, 3-dimethoxysodiumpropoxide. It was assumed that the hydrolysis would yield 2 moles of methyl alcohol to 1 mole of malonaldehyde, and that delivery of both products to the receiver would be essentially complete. An aliquot of the distillate was oxidized with excess potassium permanganate and the residual permanganate determined by titration with standard sodium thiosulfate. As-

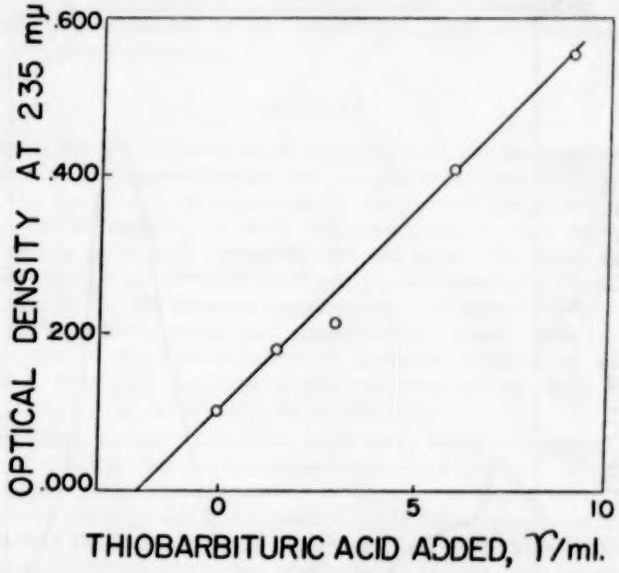

Figure 4. Results of the degradation of the malonaldehyde-thiobarbituric acid pigment in 1.5 N HCl.

suming complete oxidation of the methyl alcohol and malonaldehyde to the corresponding acids, the data permitted calculation of the malonaldehyde concentration.

Standard curves were prepared for malonaldehyde and for the primary and secondary maxima of thiobarbituric acid in 1.5 *N* HCl. A number of color solutions were analyzed for thiobarbituric acid on the basis of their absorption at the primary ultraviolet maximum. The absorption at the secondary maximum (which coincides with the malonaldehyde maximum) was determined from the graph. The residual absorption was assumed to be due to malonaldehyde. This procedure was used to determine the concentration of free thiobarbituric acid and free malonaldehyde in a number of color solutions. A plot of thiobarbituric acid concentration against malonaldehyde concentration approximated a straight line with a slope of 1.02. These data also indicate an equimolar reaction between malonaldehyde and thiobarbituric acid to produce the color compound(s).

Elemental analyses. Combustion and nitrogen analyses were performed on several pigment preparations, all of which contained inorganic material. The results were too inconsistent to justify definite conclusions. Spectrophotometric estimations of the purity of these samples were not performed.

Molecular weight determinations. Several attempts, based on colligative properties, were made to determine the molecular weight of the pigments. Due to the low solubility and slow but constant degradation these attempts were not successful.

DISCUSSION

Although the pigments prepared from thiobarbituric acid and oxidized milk fat, malonaldehyde, and 2-sulfanilamidopyrimidine exhibit the same spectral characteristics in the visible range, this observation does not justify the conclusion that the compounds are identical. As pointed out by Stearns

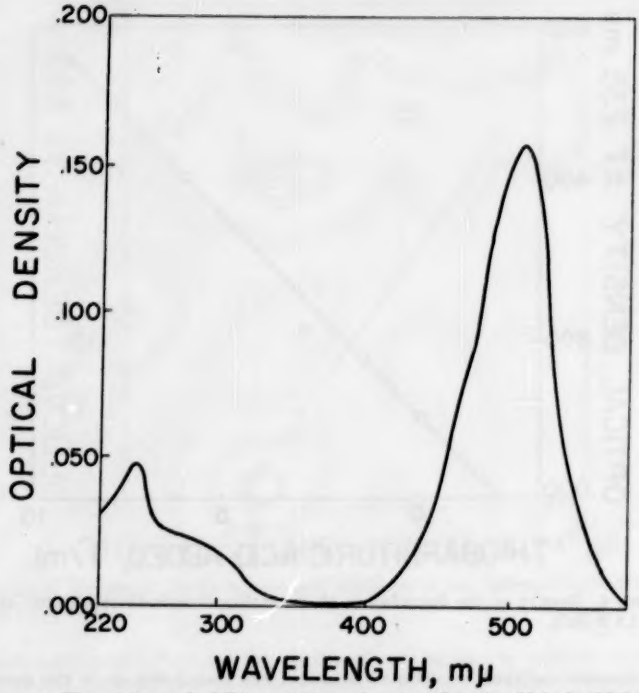

Figure 5. Ultraviolet and visible spectrum of a second malonaldehyde-thiobarbituric acid pigment.

(15), some structural differences introduce essentially no change in the spectral absorption curve over a limited range of wavelengths. Identification based on agreement of absorption curves is recognized as being at best only identification of a chromophore group.

Thiobarbituric acid reacts with aromatic aldehydes to produce highly colored precipitates. Identification of these pigments has been based principally on combustion analyses. It has been generally assumed that the pigments are relatively small molecules resulting from condensation. Attempts at characterization have been influenced by this assumption. Neither the results of this study nor data reported by others provide evidence that rules out the possibility that the pigments are larger molecules. Results presented in this paper support the observation of Kitamura and Suzuki *(8)* that pigments with different combining ratios can be formed. The presence of various reactive groups on the parent compounds could make association of condensed units possible. The formation of a relatively large complex molecule with variable composition might account for the low solubility, thermal decomposition, the failure to form derivatives, and the lack of consistency in elemental analyses.

The results reported do not justify definite conclusions regarding identity of the pigments, their structures, or the reactions by which they are

formed. Data permitting more definite conclusions concerning the molecular weights are essential before elemental analyses and combining ratios can be properly interpreted.

SUMMARY

Methods for the preparation of pigments from thiobarbituric acid and oxidized milk fat, malonaldehyde, and 2-sulfanilamidopyrimidine are presented, and the general characteristics of the pigment preparations are described. Various oxidized vegetable oils were found to react with thiobarbituric acid to produce the characteristic red color. The chromatographic adsorption techniques investigated did not prove satisfactory for isolation and purification of the pigment preparations. Attempts to form chemical derivatives were unsuccessful, and the solubility characteristics of the pigments prevented the determination of molecular weights by colligative properties. Elemental analyses of pigment preparations were not sufficiently consistent to justify definite conclusions.

A spectrophotometric technique permitting calculation of the stoichiometry of the malonaldehyde-thiobarbituric acid pigment is presented. Results suggest an equi-molar reaction without loss of water. Other spectral data indicate the pigment is degraded to yield equal quantities of malonaldehyde and thiobarbituric acid. A second malonaldehyde-thiobarbituric acid pigment with an absorption maximum of 507 mμ was isolated. The spectral techniques indicated a combining ratio of 3 aldehyde molecules to 1 thiobarbituric acid molecule for this pigment.

Acknowledgment

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PREPARATION AND UTILITY OF COLOR REFERENCES FOR DEHYDRATED SWEETPOTATOES

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Color is one of the important characteristics of a food upon which the consumer bases his initial response. If appealing, he will be encouraged to investigate further; if unnatural or abnormal, he will generally reject the product. It is therefore important to preserve as much of the original natural color as possible during processing and storage of any commodity and to check one's success by a sensory evaluation.

In an investigation of the time-temperature-tolerance of raw and dehydrated sweetpotatoes of the Unit I Porto Rico Variety, it was desirable to have the panel (11 males and 8 females selected from the scientific and administrative personnel of this laboratory) grade the reconstituted products subjectively for color. Many of the usual variables encountered in an investigation of this type were, in this case, common to all the samples. For instance, variety, climate, fertilization, and time of harvest were assumed to have had the same degree of influence on all the potatoes since the entire lot, approximately 2000 pounds, was of the Unit I Porto Rico variety, grown in West Feliciana Parish, Louisiana, and harvested in October, 1952. All handling and processing conditions were standardized and their effects on the samples were likewise assumed to be similar. Changes in color during storage, therefore, were expected to be gradual, the rate depending on the duration and temperature of storage and on the state of the sweetpotatoes, i.e., raw or dehydrated.

The Unit I Porto Rico sweetpotato has a natural bright orange color. Chemical changes occurring in the raw stock during storage have resulted in a loss of some of the pigment *(2, 9)* with the disappearance of some or most of the bright orange color giving rise to yellow orange and later yellow potatoes, depending upon the severity of the storage conditions. Production of dehydrated dice—uncooked dried sweetpotato cubes (1 cm.^{3}) —included steps which would cause only a minimum leaching of the natural color, and storage of the dehydrated product was expected to result in some browning, depending upon the temperature and time of storage among other variables *(7, 10)*.

Because of the possible chemical changes expected during storage of the raw and dehydrated sweetpotatoes, six colors arranged in the order outlined; bright orange, yellow orange, yellow, brownish yellow, brownish orange, brown were listed on the score sheet. The panel was instructed to select the term which most nearly described the color of the sample. The judges were permitted to grade a sample of the reconstituted dice (defined

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as the cooked reconstituted product made from the dehydrated dice) midway between two colors. Therefore, there were a total of 11 categories into which a sample could be placed. Color grading was deliberately coarse. However, the number of categories was sufficient to bring out trends if changes occurred since it was essential to know which temperatures contributed to or operated against the preservation of the natural bright orange color in both the raw and dehydrated material.

Judgments rendered by the panel on samples in which the carotene content and extractible color remained essentially unchanged from time of harvest and which appeared to possess a bright orange color when reconstituted received ratings indicative of much color change. Individual scores for the same sample also varied greatly. These variations might be due to the fact that the judges had no previous training in color judging and were, therefore, grading on the basis of earlier experiences totally unrelated to color grading of foods.

Available color standards were inconvenient to use and did not provide the associated wetness, gloss, and texture effects. Color plates such as those of Magoon and Culpepper *(8)* were not applicable to the problem since theirs was a varietal study which did not cover the range of colors required. As a result, it was impossible to overlook the need of the judges for color references that would parallel in appearance the wetness, gloss, and mealy texture of the pureed reconstituted dice and at the same time possess the color of the product being graded. It was believed that a simple approach to the problem could be achieved by preparing reasonably permanent references of the product embedded in plastic in a range of colors selected on the basis of color changes expected during the experiment. In using the references, color evaluation became simply a matching of colors which, according to Judd *(5)*, will succeed, provided both sample and reference are always viewed against the same surroundings.

PREPARATION OF COLOR REFERENCES

Preparation of the references involved a selection of dehydrated sweetpotato dice for uniformity of color, grinding each sample in a Wiley Mill using a 20-mesh screen, and dividing into fractions by shaking on a 20- and 40-mesh screen. The through 20 on 40 and through 40 fractions were used to prepare the references.

The bottom half of a 2-inch petri dish was filled to a depth of approximately 1.3 cm. with the monomer, methyl methacrylate (Rohm and Haas[b]) in which was dissolved 5.2% 2,4-dichlorobenzoyl peroxide (Lucidol Division, Novadel-Agene Corporation[b]) in place of the unsubstituted benzoyl peroxide recommended for embedding agricultural specimens *(3)*. A sufficient quantity of the ground dehydrated sweetpotato dice was added to the liquid in the dish so that when the solid had been thoroughly mixed and settled to an even layer, there remained a clear liquid layer of approximately 0.5 cm. in depth above the sweetpotato slurry. The dish was then placed in a pint-size, wide mouth Mason jar and the petri dish-cover set in position. Three covered petri dishes could be loaded into one jar which was sealed with a rubber-seal screw top and placed in a wooden constant temperature cabinet maintained at 100°F. (37.8°C.) for approximately 5 days. The hardened disc, usually 0.5 to 0.6 cm. thick, was obtained by breaking away the glass dish. Many were made in a variety of colors obtained from the various

[b] The mention of trade products or companies does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over similar products or companies not mentioned.

samples of dehydrated sweetpotatoes. The 6 discs approximating most closely the colors of the reconstituted sweetpotatoes described by the terms on the score sheet were mounted with putty in ⅛ inch recessions on a suitable wooden tray painted a neutral gray. Two and one-half inches of space were allowed between discs on the tray (31 × 4 × ½ inches) to permit setting the unknown contained in a 2-inch petri dish immediately adjacent to the reference. The smooth bottom of the disc was used for grading color. In appearance it resembled the moist, freshly prepared puree, the more finely ground material approximating more closely the texture of the sample to be graded. The gloss of the reference surface corresponded to that observed when the pureed sample to be graded is turned over and examined through the glass bottom of the petri dish in which it is contained. This procedure was developed with the assistance of the panel by trial and error.

Color was judged in an individual booth in which 3 walls and the ceiling were painted neutral gray. A heavy black cloth covered the entrance to the booth. Light for the color comparisons was furnished by two 100-Watt lamps placed 9 inches above the references. Unfiltered light from incandescent lamps was used in preference to daylight because the former is believed to parallel more nearly that under which the reconstituted dehydrated sweetpotatoes would be consumed.

LIFE EXPECTANCY OF REFERENCES

A slight browning became noticeable in the bright orange, yellow orange, and yellow discs after approximately 40 hours exposure to the conditions under which they were used. Therefore, it was decided to use this time interval for the best life-expectancy of the set of color references employed in these investigations. Allowing approximately 60 seconds for each judge to rate 3 samples and 20 judges to a panel, it should be possible to use the references for 120 panel sessions. Greater protection was afforded the references by covering them with a black, light-tight wooden cover between use.

OBJECTIVE MEASUREMENT OF COLOR REFERENCES

If a storage experiment is to continue in excess of 120 panel sessions, new reference standards, matching the first set as closely as possible are a prime requisite. Furthermore, if grading of color of reconstituted dehydrated sweetpotatoes by the subjective method outlined, is to be conducted in more than one laboratory, references which match very closely will be required if equivalent grading is to be expected from the color panels. It therefore becomes desirable to obtain objective color designations, in accepted color terms, of the references used which will permit their duplication at any future time or at any other location.

A spectral reflectance curve, in terms of optical densities, was obtained for each of the 6 references by use of a Cary automatic recording quartz spectrophotometer[b] equipped with a diffuse reflectance attachment. From these curves optical densities were read at 10 mμ intervals and converted to reflectance. Tristimulus values and the trichromatic coordinates x, y, and z, were calculated by the weighted ordinate method using data for the C. I. E. illuminant C and the C. I. E. standard observer from Table 1 in a paper by Kelly, Gibson, and Nickerson *(6)*. The psycho-physical attributes, dominant wavelength, and excitation purity, were determined by application of the tristimulus value Y and the trichromatic coordinates x and y to Charts Nos. 13 and 18 in the *Handbook of Colorimetry (4)*. Luminous directional reflectance is equal to Y multiplied by 100. Munsell renotations were obtained by application of the C. I. E.-Munsell conversion charts in a paper by Newhall, Nickerson, and Judd *(11)*. These values for the 6 references are given in Table 1.

The trichromatic value Y, on the equivalent luminous directional reflectance, is low for all standards. As might be predicted, the x trichromatic coordinate, or "red factor," increases from the yellow to the bright orange reference, then decreases toward the brown. The dominant wavelength exhibits a small but steady shift toward longer wavelengths from the yellow through the bright orange to the brown reference. These values, either C. I. E. designations or Munsell renotations, constitute data from which the 6 references can be reproduced exactly at any time or at various locations.

Although not of primary importance to a subjective method for grading color, it was of some interest to compare objective color values for the references with some

TABLE 1

C. I. E. color designation and Munsell renotations of sweetpotato color references and of representative reconstituted dehydrated sweetpotato samples

Color name	Tristimulus value Y	Trichromatic coordinates		Psycho-physical attributes			Hue	Value/Chroma
		x	y	Reflectance	Dominant wave length	Excitation purity		
References								
				%	mμ	%		
Yellow	0.06188	0.3792	0.3714	6.19	581	33.2	1.3Y	2.9/2.2
Brownish yellow	0.04246	0.4061	0.3885	4.25	582	45.1	1.0Y	2.4/2.8
Orange yellow	0.03922	0.4149	0.3766	3.92	585	44.2	8.4YR	2.3/2.8
Bright orange	0.04081	0.4538	0.3892	4.08	587	58.1	7.3YR	2.3/3.9
Brownish orange	0.04164	0.4213	0.3722	4.16	587	44.9	6.8YR	2.4/3.0
Brown	0.01786	0.3804	0.3382	1.79	593	24.4	4.1YR	1.4/1.4
Samples								
Yellow	0.06726	0.4285	0.4140	6.73	580	58.0	2.2Y	3.0/4.0
Brownish yellow	0.07194	0.4292	0.4141	7.19	580	58.2	2.0Y	3.1/4.1
Orange	0.05899	0.4543	0.4162	5.90	583	65.2	0.5Y	2.8/4.6
Brownish orange	0.06295	0.4541	0.4183	6.30	582	65.9	0.1Y	2.9/4.7
Brown	0.05554	0.4502	0.4124	5.55	583	63.3	0.3Y	2.8/4.4

actual reconstituted dehydrated sweetpotato samples. C. I. E. designations and Munsell renotations were obtained in exactly the same manner as these data had been measured for the references. The values for 5 sweetpotato samples, covering most of the range of the color references are included in Table 1. As the references were made from selected dehydrated sweetpotato dice, the trichromatic value Y or luminous directional reflectance might reasonably be expected to be closely similar for both references and sweetpotato samples. As selection of the references was made by a visual examination of the samples, the x coordinate, or "red factor" to which the eye is particularly sensitive, might also be predicted to be in close agreement between sample and reference. Data in Table 1 indicate that for a sample and a corresponding reference these values are similar. The reflectance is of the same low order of magnitude as the references for all samples and the x coordinate for any sample has a value very close to the corresponding reference showing the same increase in value from yellow to bright orange and subsequent decrease to brown. The dominant wavelength shows the same tendencies to shift to longer wavelengths and is of the same general magnitude for samples and references.

In matching color, however, the eye may substitute for a reflectance at a particular frequency, reflectances at other frequencies which will result in close visual match under one illumination. Hence, references and samples may be metamerie and values for excitation purity and value/chroma ratio, which depend upon spectral isolation, may vary considerably. Data in Table 1 show that in the samples measured these values differ considerably. However, in a subjective method of color grading, these differences are of no great concern as long as the conditions of illumination under which the matching is to be accomplished are critically defined and maintained.

UTILITY OF COLOR REFERENCES

Utility of the references resulted in a redistribution of judgments, more nearly in accord with the appearance and history of the samples when all samples being graded had approximately the same carotene content. Three samples stored as raw stock for one month at 50° (10°C.), 60° (15.6°C.), and 70°-75°F. (21.1-23.9°C.), were dehydrated, and found to contain about the same quantity of carotene, Table 2. These samples were reconstituted and graded without references, 19 judgments being

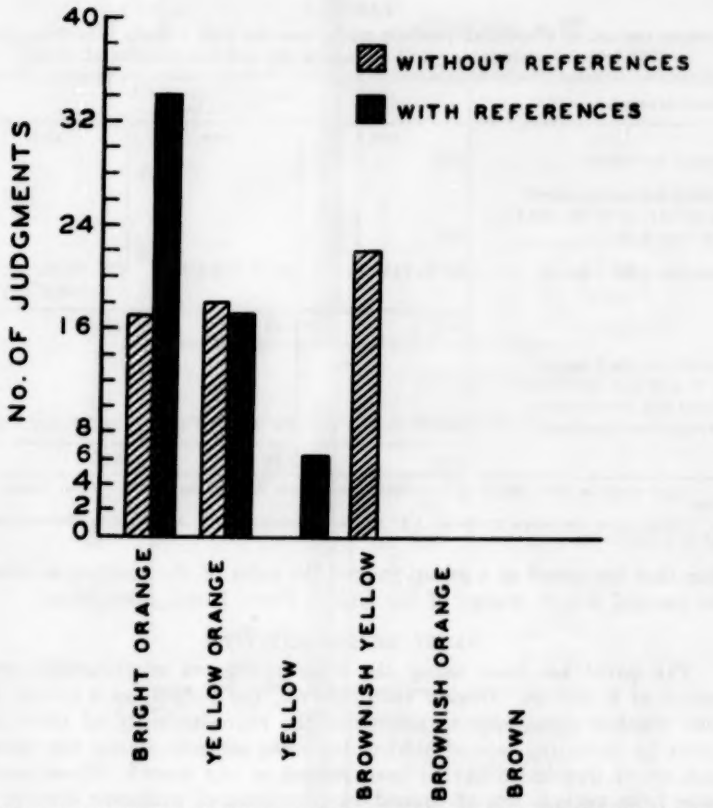

Figure 1. Distribution of fifty-seven judgments of color with and without references.

obtained for each sample. Since all 3 samples contained approximately the same quantity of carotene, all judgments were grouped and treated as those of one sample. Distribution of the 57 judgments is illustrated by the striped bars in Figure 1. The histogram shows that less than ⅓ of the judges rated the sample as bright orange, approximately ⅓ rated it as yellow orange, and the remainder thought it was brownish yellow. It was interesting to note that all the judges avoided the term yellow.

The raw sweetpotatoes stored for one month at 50°F. (10°C.) were dehydrated, subdivided, and subsamples were stored under nitrogen at −10° (−23.3°C.), 75° (23.9°C.), and 100°F. (37.8°C.). The carotene content of these samples was essentially the same and showed no decrease as compared to the parent sample or to the freshly harvested and cured potatoes (Table 2). Graded with references, redistribution of the 57 judgments is illustrated by the black bars in Figure 1. Almost ⅔ of the judges rated the samples as bright orange, nearly ⅓ as yellow orange and an insignificant few as yellow. With the aid of color references it is shown

TABLE 2

Carotene content of dehydrated products made from the Unit I Porto Rico Sweetpotato, 1952 crop, at various stages of storage in the raw and dehydrated state.

History of sample[1]	Carotene content[2] on a dry wt. basis		
	ppm	*ppm*	*ppm*
Freshly harvested	203		
Freshly harvested, cured for 10 days at 85°F. (29.4°C.) and 85% R.H.	229		
Stored raw for 1 mo. at	50°F. (10°C.)	60°F. (15.6°C.)	70°-75°F. (21.1-23.9°C.)
	217	226	252
Stored raw for 1 mo. at 50°F. (10°C.), then dehydrated and stored under nitrogen for 9 weeks at	-10°F. (-23.3°C.)	75°F. (23.9°C.)	100°F. (37.8°C.)
	266	224	224

[1] Raw products were stored at Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

[2] Each figure represents the average of duplicate determinations, according to alternate method (b) of A.O.A.C. *(1)*.

then that the panel as a group judged the color of the samples as close to the natural bright orange of the Unit I Porto Rican sweetpotato.

PANEL REPRODUCIBILITY

The panel has been using the color references continuously over a period of 6 months. During that interval, the judges, as a group, have been checked repeatedly to determine the reproducibility of their judgments by including sets of hidden duplicate controls among the samples, each set of duplicates having been judged at one session. These controls came from various lots of canned sweetpotatoes of unknown history. Results representative of panel reproducibility in judging color with the aid of references are illustrated in Figure 2, giving data for 3 different sets of hidden duplicate controls. In this figure, each letter signifies that a different control sample had been used. The subscripts indicate that 2 samples were presented from each control. Since these were hidden controls, they were coded by labelling with 2 different letters when presented to the panel so that their relationship remained unknown. The judgments rendered for one set of duplicate controls are represented by the shaded and unshaded bars. For example, the shaded bar in the topmost histogram of Figure 2, represents the summation of judgments rendered for Control A_1. The unshaded bars represent the same number of judgments for Control A_2. There is only a slight variation in the pattern. The histograms, demonstrating the ability of the panel to rate duplicates, have been arranged in chronological order reading from the top down.

DISCUSSION AND SUMMARY

Color evaluation of foods is extremely important as has been pointed out and instrumental determinations of color do not take into account

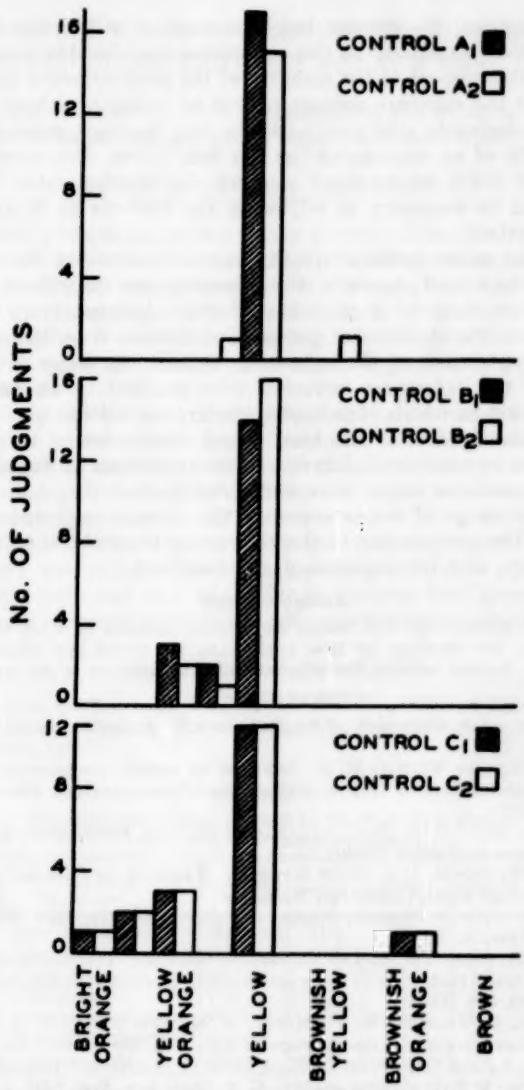

Figure 2. Reproducibility of color judgments with the aid of color references.

the psychological aspect of the problem. Color dictionaries contain so many shades of color that any attempt to use them only adds to the confusion. Specially made tiles even though portraying the exact shades desired lack the appearance of the product being graded. The utility of the references described here suggest that the idea may have wide application to many other agricultural products, particularly in the fruit and

vegetable industries. No attempt has been made to utilize this idea in the case of other food materials. In this connection considerable caution should be exercised with respect to the stability of the product being investigated, particularly if the moisture content cannot be reduced without the development of undesirable side reactions. In this instance moisture content was apparently of no consequence as the dehydrated dice were very dry, approximately 5-6% in moisture content. It is conceivable that many variations will be necessary in adjusting the formula to fit the product being investigated.

Use of color as an index of quality is fundamental in the reaction of the consumer to a food; however, the measurements made here should not be construed as those of consumer preference because every effort was made to remove the elements of personal preference from the actual color judgment. Application of the references limited the scope of judging to the range of the references reducing color-grading to an act of color-matching, an act in which, it is hoped, preference did not participate.

In this investigation it has been found advantageous to grade color subjectively in reconstituted dehydrated sweetpotatoes by furnishing plastic color-references in which were embedded ground dehydrated sweetpotato dice in a range of colors covering the changes expected during the experiment. The preparation of the references, their objective color designations, utility, and life-expectancy are described.

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THE NUTRITIVE VALUE OF LEGUME PROTEINS AND LEGUME-WHEAT MIXED PROTEINS IN NEAR EAST DIETS

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Near East peoples follow essentially a cereal diet *(2)*. Meat and other animal protein foods are included only in small amounts. In some rural areas the amount of meat consumed is almost *nil* except for occasional indulgence on feast days. The cereal diet, however, is usually supplemented with liberal amounts of legumes. Our experience suggests that such legume-cereal mixtures supply a mixed protein which is often of superior nutritive value.

We are reporting here protein efficiency ratios measured by the rat growth method on the commonly used legumes, and also on the widely used parboiled wheat of the Near East. Included are measurements on a group of legume and parboiled wheat mixtures of which at least three are in common use in Near East areas. These mixtures were composed in each case of the dried materials mixed in 1:1 and 1:1:1 proportions (Table 3). Whole wheat was included in the series as a reference material. These measurements were part of a search for indigenous food materials containing high quality protein suitable for milk substitutes in infant feeding.

EXPERIMENTAL PROCEDURE

Table 1 gives the proximate analysis for market samples of legumes and wheat products purchased in Beirut. The methods of analysis were those of the A.O.A.C. (ref. 7th edition, 1950).

TABLE 1

Proximate analysis of legumes in the Near East dietary
(In per cent of edible portion)

English name	Botanical name	Arabic name	Moisture	Protein (N × 6.25)	Fat	Available carbohydrate	Ash
Chick pea	*Cicer arietinum*	Hommos	11.8	17.8	5.2	59.2	2.6
Lupine	*Lupinus termis*	Turmus	10.0	36.8	9.4	11.3	2.9
Cowpea	*Vigna catiang*	Lubiya	11.9	19.6	1.1	48.4	3.1
Broad bean	*Vicia faba*	Ful	10.1	23.5	1.2	59.9	2.7
Scarlet bean	*Phaseolus multiflorus*	Fasulia	11.9	19.6	1.5	40.2	3.5
Kidney bean	*Phascolus vulgaris*	Fasulia	11.6	22.5	0.9	45.0	3.5
Lentil	*Lens esculenta*	Adas	10.8	24.0	0.8	50.7	2.6

The protein efficiency ratio has been determined by standardized technique, using weanling rats, averaging about 40 g. in weight. Measurements are reported on 13 different diets, with 10 animals as a rule on each diet. Litter mates and males and females were distributed as evenly as possible between the different diet groups. The animals were fed a uniformly controlled intake, each rat being fed 7 g. per day at the beginning of the experiment, which was increased after one week to 8 g., then to 9 g. and finally to 10 g. for the fourth and last week. These graded amounts represented a border-line intake based on earlier experiments with the paired feeding method. In a few cases, the actual intake fell slightly below the amounts fed. The animals were

housed in individual screen-bottom cages. The usual precautions were taken; non-scattering food cups were used. Distilled water was available *ad libitum*. The intake was measured daily and the animals were weighed at 3-day intervals. An adjustment period of 2 days was allowed at the beginning of the experiment; the measurements proper covered a total of 30 days.

Each of the diets was made up to contain the food material being studied as the sole source of protein, on a 10% protein level. The diets were uniformly constructed and were essentially equicaloric except for the slight error caused by qualitative differences in the carbohydrate present in the diet. Each diet contained 2% of cod liver oil; the total fat content was equalized at a 10% level by adding hydrogenated fat (CRISCO). Each diet also contained 2% of salt mixture *(4)* and 2% of dextrose containing the vitamin mixture recommended by Horn *et al (3)*, but to which vitamin B_{12} was added at a level of 2.5 micrograms per kilo of diet. Finally, corn starch was added to bring the total constituents of each diet to 100%.

The vitamin mixture employed was mixed with dextrose in such amount that the desired amounts of the vitamins were supplied when the dextrose base as noted above was added so as to constitute 2% of the diet. Amounts of each diet less the dextrose-vitamin mixture were prepared sufficient for the entire period of the experiment, and the protein content ($N \times 6.25$) checked by Kjeldahl determination. To avoid loss of vitamin potency in the mixed diet, the vitamin-dextrose mixture was prepared separately and incorporated into amounts of diet sufficient for 7-8 days of feeding.

In the case of the legumes tested, cooked rather than uncooked material was used. The method of cooking was that commonly employed in the Near East, which involved simply adding water and then cooking till soft; this required from one to two hours. The chick peas and broad beans were first soaked in water overnight before cooking. In the case of chick peas, about 10 g. of sodium bicarbonate per kg. was added during the cooking process. The cooked material was then rapidly dried in a forced-draft oven at 65°C., powdered, and incorporated into the diets in this dried form; parboiled wheat was not submitted to further cooking. All legume and parboiled wheat samples before incorporation into diets were milled to pass 20 mesh. Included in the measurements was one group of animals fed uncooked chick pea (Table 2).

TABLE 2

Protein efficiency of legumes and wheat products used in the Near East

(Ten per cent protein level; experimental period: 30 days; 10 rats in each group, except as indicated. Protein efficiency ratio = grams of growth per gram of protein consumed)

Protein-containing food material	Average gain in weight	Protein intake	Protein efficiency ratio ± standard deviation
Legumes	*grams*	*grams*	
Chick pea[1, 2]	30.4	20.6	1.47 ± 0.24
Chick pea, cooked	49.1	23.9	2.05 ± 0.10
Lentils, cooked	25.7	22.1	1.15 ± 0.14
Broad bean, cooked	30.8	26.0	1.17 ± 0.17
Kidney bean, cooked	36.9	24.3	1.51 ± 0.22
Wheat Products			
Parboiled wheat *(burghul)*	32.6	22.5	1.44 ± 0.07
Wheat[1]	31.8	22.8	1.39 ± 0.20

[1] Uncooked.
[2] Six rats.

RESULTS AND DISCUSSION

The results of the protein efficiency measurements are shown in Tables 2 and 3; the protein efficiency ratio is calculated in the usual manner as the growth in grams per gram of protein consumed. Among the single legumes (Table 2) the highest value is that shown by the chick pea. It is

significant that this is the one most widely used among the legumes in the Near East; it is usually eaten in the form of a cooked gruel mixed with sesame emulsion. The uncooked chick pea shows a much lower protein efficiency ratio. The broad bean, extensively used in Egypt, shows a low protein efficiency ratio. Parboiled wheat as used in the Near East has been described elsewhere *(5)*.

TABLE 3

Protein efficiency of mixed protein food materials

(Ten per cent protein level; experimental period: 30 days; 10 rats in each group. Protein efficiency ratio = grams of growth per gram of protein consumed)

Protein-containing food material		Average gain in weight	Protein intake	Protein efficiency ratio ± standard deviation
		grams	*grams*	
Chick pea* + Parboiled wheat	(1)	56.4	27.0	2.10 ± 0.26
Lentils* + Parboiled wheat	(1)	37.5	23.9	1.55 ± 0.24
Broad bean* + Parboiled wheat	(1)	42.0	25.7	1.62 ± 0.12
Broad bean* + Chick pea*	(1)	43.6	29.9	1.49 ± 0.19
Broad bean* + Chick pea* + Parboiled wheat	(2)	53.0	27.3	1.94 ± 0.14
Parboiled wheat + Milk *(leban)*	(3)	50.0	25.8	1.93 ± 0.19

* Cooked.
(1) mixture 1:1 by weight.
(2) *Falafil:* mixture 1:1:1 by weight.
(3) *Kishk:* consisting approximately of two parts of parboiled wheat and one part of milk *(leban)* by weight.

The protein efficiency ratios for the mixtures (Table 3) indicate that the addition of parboiled wheat to any of the single legumes yields a mixture with increased nutritive value in respect to the protein fraction. The chick pea and parboiled wheat are two food materials that frequently appear together at the same meal, although they are not usually combined in a prepared mixed cereal. The protein efficiency ratio of this chick pea-parboiled wheat mixture is high.

The mixture containing the two legumes plus parboiled wheat in the proportion 1:1:1 is the material used in making *falafil*, a coarse meal-mush, which is cooked by deep-frying and is very popular among the laboring class in Lebanon; it is regarded, however, as a second class food. Its protein efficiency ratio is shown here to be very high. *Kishk (2)*, made from parboiled wheat plus *leban (yoghurt)*, is essentially milk dried on a parboiled wheat base; it is a food material long in use in the eastern Mediterranean area and is regarded as of high nutritive value. It shows a high protein efficiency ratio.

We have previously reported the use of legume-cereal mixtures in North China *(1)* and have stressed the superior nutritive value of some of these mixed protein sources. It would appear that rural peoples whose food intake is limited essentially to food materials of vegetarian origin have often, perhaps by blind experiment, selected for use just those mixtures where the protein fraction, as shown by subsequent laboratory experiment, possessed a strikingly high nutritive value.

SUMMARY

Measurements of the protein efficiency ratio have been carried out on the common legumes used in the Near East and on various legume-containing mixtures, in which parboiled wheat is employed as one of the ingredients.

Cooked chick pea alone, and the cooked chick pea-parboiled wheat mixture, both are shown by this method to possess high nutritive value with respect to the protein. The widely used *falafil*, a chick pea-broad bean-parboiled wheat mixture, also shows a high protein efficiency. Some degree of supplementary action among the protein constituents is evident for all the mixtures examined.

These measurements suggest the important role of mixed food protein sources in areas where the intake is largely restricted to proteins of vegetarian origin.

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THE EFFECT OF BRAISING AND PRESSURE SAUCEPAN COOKERY ON THE COOKING LOSSES, PALATABILITY, AND NUTRITIVE VALUE OF THE PROTEINS OF ROUND STEAKS[a, b]

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Widespread interest in the pressure saucepan for preparing the less tender cuts of meat led to a study of the influence of certain modifications of this procedure upon the cooking losses, palatability, and nutritive value of the proteins of round steaks. Few reports comparing the cooking losses and palatability of beef cooked by braising with that cooked under pressure have been published. Ehrenkranz and DeAtley *(3)* reported that when 14 beef round roasts from one animal were cooked to an internal temperature of 175°F. (79.4°C.) in a conventional saucepan on top of a stove, total cooking losses averaged 28.65%, and those for 6 roasts from another animal averaged 30.18%. Roasts cooked under 10 p.s.i.g. pressure lost an average of 33.69% of their weight. Tucker, Hinman, and Halliday *(12)* obtained an average weight loss of 36% for heel of round roasts braised on top of a stove, 33% for those braised in an oven, and 34% when cooked under 15 p.s.i.g. pressure to an internal temperature of 199.4°F. (93°C.). Clark and Van Duyne *(2)* studied the effects of roasting top round of beef at 300°F. (148.9°C.) to an internal temperature of 179.6°F. (82°C.), and of cooking this cut of meat under 15 p.s.i.g. pressure to the same internal temperature. The roasted meat lost an average of 29.5% of its original weight, whereas the pressure cooked meat lost an average of 35.6%. There was no difference in the tenderness of the meat cooked by the 2 methods, but a palatability panel preferred the flavor of the roasted meat and considered the pressure cooked meat too dry.

The influence of heat upon the nutritive value of meat proteins has been reviewed recently by Cannon *(1)*, Griswold *(4)*, and Rice and Beuk *(10)*. Rice and Beuk postulated that a certain critical temperature range, probably around 100°C. (212°F.), must be exceeded before biologically detectable heat damage occurs. Roasting to an internal temperature of 160°F. (71.1°C.) did not reduce the biological value of beef proteins tested by Mitchell, Hamilton, and Beadles *(8)*. Moreover, Kuiken and Lyman *(6)* observed that the essential amino acids of roast beef were completely available; and Neilands, Sirny, Sohljell, Strong, and Elvehjem *(9)* concluded that the essential amino acid composition of meat or fish was not altered materially during commercial canning.

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The present study was designed to compare meat cooked in a pressure saucepan under specific conditions with meat braised at atmospheric pressure in respect to: *(a)* cooking losses, *(b)* palatability, and *(c)* nutritive value of the proteins.

EXPERIMENTAL METHODS

Paired rounds from 3 carcasses, graded U.S. Commercial, were purchased from a Kansas City packing house. The 6 rounds were divided into top and bottom portions, and 4 steaks 1.5 inches thick were cut from each top and bottom round. Steaks from the left and right sides of the carcasses were cut as pairs. Each piece of meat, wrapped in cellophane with an outer wrap of waxed locker paper, was frozen in a blast freezer at −10°F. (−23.3°C.), and stored at 0°F. (−17.8°C.) until used.

Cooking methods. Three-fourths of the steaks were cooked in a pressure saucepan, and were equally divided among 3 cooking conditions: (a) under 10 p.s.i.g pressure to an internal temperature of 176°F. (80°C.), (b) under 15 p.s.i.g. pressure to an internal temperature of 176°F. (80°C.), and (c) under 15 p.s.i.g. pressure to an internal temperature of 234°F. (112.2°C.). The remaining one-fourth of the steaks were braised to an internal temperature of 176°F. (80°C.) in a rotary gas oven maintained at 300°F. (148.9°C.). A randomized complete block design was used for assigning the cooking methods to the steaks. Four adjacent paired steaks, each given a different treatment, i.e., method of cooking, made up a block. Analyses of variance were run on flavor, tenderness, and juiciness scores, shear values, and press fluid yields for the top and bottom round steaks.

The frozen steaks were thawed in a refrigerator for 48 hours prior to cooking, then browned for 7.5 minutes on each side in 20 g. of suet. The steaks to be cooked under pressure were placed on a rack in a pressure saucepan (containing 30 ml. of water) equipped with both a thermometer and a thermocouple. The thermocouple was placed in the center of the *semimembranosus* muscle of the top round steaks or in the *biceps femoris* muscle of the bottom round steaks and the cover of the pressure saucepan adjusted. The thermocouple was then attached to a Leeds and Northrup potentiometer which gave a direct reading in degrees Fahrenheit. The steam was allowed to escape from the saucepan for one minute before the weight was placed on the vent.

When the steaks cooked to 176°F. (80°C.) reached this temperature, the pressure saucepan was removed from the heat, the pressure released immediately, and the meat removed from the pan. Steaks cooked to 234°F. (112.2°C.) remained in the saucepan after it was removed from the heat until the pressure returned to zero, which required 10 minutes.

The steaks to be braised were placed on a rack in an enamel roaster. A thermometer was inserted in the meat in the same position as described for the thermocouple. Thirty ml. of water was added, the roaster was covered and placed in an oven maintained at 300°F. (148.9°C.), and the meat cooked to an internal temperature of 176°F. (80°C.).

Palatability tests. A panel of 7 judges scored the meat for aroma, flavor of the lean, tenderness, and juiciness. Scores for each factor were given within the range of 10 to 1. Tenderness was scored on the basis of the number of chews required to completely masticate a bite of meat of standard size. Samples prepared by each of the 4 methods of cooking were tasted at one time. The meat was tasted cold since it was possible to pressure cook only one steak at a time. Each time the steaks were tasted, the judges stated a preference for one of them.

Objective tests. Thawing losses, cooking time, cooking losses, shear values, and press fluid yields were determined for each steak. One-inch cores of meat were sheared on the Warner-Bratzler shearing apparatus and 25-g. samples of ground meat were pressed in the Carver laboratory press.

Measurement of nutritive value. The nutritive value of the meat prepared by the 4 methods under test and of the uncooked meat was evaluated in terms of growth and retention of nitrogen by weanling male rats of the Sprague-Dawley strain. Animals with an initial weight of 54 ± 3 g. were distributed randomly among the 5 groups, 8 or 10 rats being assigned to each. Analysis of variance and covariance were used to determine whether significant differences existed among the daily gains, nitrogen efficiencies, nitrogen balances, and quantities of nitrogen stored in livers and carcasses of the rats in the respective groups.

All portions of the uncooked rounds that were not required for cooking were pooled to provide raw meat for a reference group. Samples from specific areas in each steak given a particular treatment were utilized for investigation of the influence of that treatment upon the nutritive properties of the proteins. The 4 samples of cooked steak and one of raw meat were ground twice, using the medium blade of the grinding attachment of an electric mixer. Layers of ground meat, approximately 1 inch thick, were dried before an electric fan at room temperature for 2 hours, then dehydrated in a vacuum oven for 3 hours at a temperature not exceeding 140°F. (60°C.), and extracted with ether in a Soxhlet apparatus of semicommercial size.

The 5 lots of defatted, dehydrated beef were incorporated in the experimental diets in amounts that provided 1.6% of nitrogen in 5 rations and 2.0% in 5 others. Although 10% of protein, i.e., approximately 1.6% of nitrogen, is frequently selected as the most suitable proportion for test purposes, Hoagland, Ellis, Hankins, and Snider *(5)* reported that a diet containing beef in this concentration was inadequate for the growing rat in both of the sulfur-containing amino acids, and that a ration supplying 12.5% of protein was somewhat deficient in cystine. Therefore, it seemed advisable to determine whether differences in nutritive value related to the cooking processes could be detected when either 1.6 or 2.0% of nitrogen from beef was consumed. The diets also contained 20% Crisco, 2% Ruffex, 5% Richardson and Hogan salt mixture *(11)*, 5% vitamin mixture, and dextrin to total 100%.

The dry vitamins, which were dispersed with dextrin, were present in mg. per kg. of diet as follows: ascorbic acid, 100; biotin, 0.1; choline, 500; folacin, 0.6; inositol, 1000; niacin, 50; para-aminobenzoic acid, 1000; pantothenic acid, 10; pyridoxine, 4; riboflavin, 6; and thiamine, 4. Each kg. of diet contained 75 mg. of alpha versus σ-tocopherol acetate. Preliminary tests indicated that gains in weight were equally satisfactory whether these vitamins were incorporated in the ration or offered as a separate supplement. In addition, Squibb Navitol with Viosterol supplied 1666 I.U. of vitamin A and 333 I.U. of vitamin D orally per week.

The diets were offered *ad libitum* for 28 days. During the last 7 days of the experiment, urinary and fecal collections were made. Urine was collected on acid-washed filter papers, one of which was transferred daily to a 20% solution of hydrochloric acid. The individual solutions then were removed by suction and made to an appropriate volume. Feces were freed from hair and dropped daily into a 20% solution of hydrochloric acid. Ferric oxide was used as a marker. Food was withdrawn for 8 hours before the initiation and conclusion of the balance period and again before the rats were killed. The animals were sacrificed 24 hours after the balance period was terminated. Livers were weighed and covered with a 20% hydrochloric acid solution. Carcasses from which the intestinal contents had been removed were covered with concentrated hydrochloric acid, 100 ml. of acid being used for rats weighing 150 g. or less, and 150 ml. for heavier rats. The flasks containing the carcasses were rotated at intervals for 7 days; then water was added to dilute the acid to approximately 20%. Livers, carcasses, and feces were autoclaved in loosely covered flasks. The solutions were poured through a sieve and diluted to suitable volumes. Nitrogen was determined by the macro-Kjeldahl method with mercuric oxide as the catalyst.

RESULTS AND DISCUSSION

Cooking time and cooking losses. Average cooking time and cooking losses for the top and bottom round steaks are given in Table 1. The average time, in minutes per pound, required to cook the braised steaks to an internal temperature of 176°F. (80°C.) was from 3.5 to 4.5 times longer than that required by steaks cooked to the same internal temperature in the pressure saucepan, and from 1.6 to 2.1 times longer than steaks cooked to 234°F. (112°C.). In general, bottom round steaks required more time per pound to cook than top round steaks.

Total cooking losses of the steaks cooked to 176°F. (80°C.) by braising and cooking under 10 and 15 p.s.i.g. pressure were practically the same. The losses of top round steaks cooked to 176°F. (80°C.) averaged 33.5%,

whereas those for bottom round steaks averaged 29.7%. Average cooking losses for top and bottom round steaks cooked under 15 p.s.i.g. pressure to 234°F. (112°C.) were 40.9 and 40.5%, respectively. Cooking losses for the steaks cooked to 234°F. (112°C.) were significantly greater ($P < .01$) than those for the steaks cooked to 176°F. (80°C.).

TABLE 1

Average cooking time, cooking losses, palatability scores, shear values, and press fluid yields

Cut	Factor	Treatment			
		Braised to 176°F.	10 p.s.i.g. pressure to 176°F.	15 p.s.i.g. pressure to 176°F.	15 p.s.i.g. pressure to 234°F.
Top round steaks	Cooking time, min./lb.	19.2	5.3[1]	5.7[2]	9.1[3]
	Cooking losses, %	33.1	33.1	34.3	40.9**
	Aroma[4]	7.5	7.4	7.6	7.1
	Flavor[4]	7.1	7.2	7.0	6.0**
	Tenderness[4]	6.3	6.1	6.2	7.1*
	Shear, lb.	25.3	22.7	23.3	19.7*
	Juiciness[4]	5.6	5.7	5.0	2.9**
	Press fluid, ml./25 g.	7.0	6.3	6.2	4.5**
Bottom round steaks	Cooking time, min./lb.	23.9	6.3	5.1	14.6
	Cooking losses, %	29.3	29.9	29.8	40.5**
	Aroma[4]	7.4	7.5	7.4	6.9
	Flavor[4]	7.2	6.9	6.9	5.7**
	Tenderness[4]	6.3	6.8	6.8	8.6**
	Shear, lb.	19.9	19.4	19.6	14.8
	Juiciness[4]	5.8	5.0	5.2	2.8**
	Press fluid, ml./25 g.	6.7	6.2	5.8	3.3**

[1] Plus 6 minutes for pressure to reach 10 p.s.i.g.
[2] Plus 7 minutes for pressure to reach 15 p.s.i.g.
[3] Plus 7 minutes for pressure to reach 15 p.s.i.g. and 10 minutes for it to return to 0 p.s.i.g.
[4] Highest possible score, 10
* Significant at the 5% level.
** Significant at the 1% level.

Palatability. Average palatability scores, shear values, and press fluid yields for the steaks are presented in Table 1. In general, the palatability of top and bottom round steaks was similar. However, bottom round steaks (*biceps femoris* muscle) were slightly more tender, as measured by tenderness scores and shear values, than top round steaks (*semimembranosus* muscle).

There was little difference in the palatability of the steaks cooked to an internal temperature of 176°F. (80°C.) by braising and those cooked under 10 or 15 p.s.i.g. pressure, but analyses of variance showed significant differences in the flavor, tenderness, and juiciness of these steaks and the same factors for the steaks cooked under 15 p.s.i.g. pressure to 234°F. (112°C.). On the average, steaks cooked to 234°F. (112°C.) scored lower in aroma (0.3 to 0.6 points), flavor (1 to 1.5 points), and juiciness (2.1 to 3.0 points), but higher in tenderness (0.8 to 2.3 points) than those cooked to 176°F. Shear values and press fluid yields agreed with the tenderness and juiciness scores. Thus, the internal temperature to which the meat was cooked, rather than method of cooking, was important in determining the palatability as well as the cooking losses of the steaks.

Nutritive value. The data pertaining to average intakes and retentions

TABLE 2

Average food intakes, gains in weight, and quantities of nitrogen retained by rats

	Treatment				
	Braised to 176°F.	10 p.s.i.g. pressure to 176°F.	15 p.s.i.g. pressure to 176°F.	15 p.s.i.g. pressure to 234°F.	Raw
	1.6% nitrogen in diet				
Food eaten daily for 28 da., g.	9.3	9.4	9.7	9.5	10.3
N eaten daily for 28 da., mg.	156	156	161	158	159
Daily gain, g.	3.3	3.2	3.5	3.3	3.6
Gain/g. N eaten, g.	20.9	20.5	21.6	21.0	21.4
Body wt. in balance period, gm.	136	136	140	137	143
Surface area, sq. cm.[1]	238	238	243	239	246
N eaten in 7-day balance test, mg./100 sq. cm.	498	500	518	520	558
N in urine, mg./7 da./100 sq. cm.	127	127	125	153	149
N in feces, mg./7 da./100 sq. cm.	75	74	80	81	87
N balance, mg./7 da./100 sq. cm.	296	297	313	286	322
Wt. of fresh liver, g.	5.37	5.24	5.77	5.42	5.31
Total N in liver, mg.	141	149	146	150	151
Total N in carcass, g.	3.89	3.83	3.97	3.92	4.10
	2% nitrogen in diet				
Food eaten daily for 28 da., g.	10.9	11.7	11.6	11.8	11.6
N eaten daily for 28 da., mg.	219	234	240	242	237
Daily gain, g.	4.8	5.3	5.3	5.3	5.1
Gain/g. N eaten, g.	21.7	22.5	22.3	21.3	21.5
Body wt. in balance period, g.	168	181	180	179	175
Surface area, sq cm.[1]	271	283	283	282	277
N eaten in 7-day balance test, mg./100 sq. cm.	704	686	718	725	710
N in urine, mg./7 da./100 sq. cm.	162	171	173	204 **	174
N in feces, mg./7 da./100 sq. cm.	79	97	98	99	88
N balance, mg./7 da./100 sq. cm.	442	417	447	423	446
Wt. of fresh liver, g.	6.55	7.27	6.93	7.68*	6.71
Total N in liver, mg.	182	202	200	222 **	198
Total N in carcass, g.	5.04	5.49	5.41	5.39	5.34

[1] Calculated by Lee formula, *Am. J. Physiol.*, **59**, 24 (1922).
* Significant at the 5% level.
** Significant at the 1% level.

of nitrogen by weanling rats fed 1.6 and 2.0% of nitrogen from each of the 5 lots of beef are presented in Table 2.

When 1.6% of nitrogen was incorporated in the rations, the average quantities of diet eaten daily by the rats fed the 5 experimental diets ranged from 9.3 to 10.3 g., but did not differ significantly as shown by analysis of variance. Average daily intakes of nitrogen during the 28-day interval also were strikingly similar, varying only from 156 to 161 mg. Neither the daily increments in weight, 3.2 to 3.6 g., nor the nitrogen efficiencies, 20.5 to 21.4, differed significantly. Homogeneity in respect to nitrogen balance was revealed by correlation analyses. Moreover, the weights of fresh livers and the amounts of nitrogen present in both livers and carcasses were similar in all groups. Since the responses of all 4 groups receiving 1.6% of nitrogen from cooked meat were similar to those obtained when uncooked beef was fed, it may be concluded that none of these methods of preparation caused a reduction in the nutritive properties of the proteins

which could be measured when a quantity of beef that permitted good but not optimum growth was fed.

When the diets provided 2.0% of nitrogen, the rats in the various groups consumed slightly larger quantities of food than when 1.6% of nitrogen was allowed, i.e., 10.9 to 11.8 g., and ingested on the average 219 to 242 mg. of nitrogen daily. Again, no significant differences existed in respect to daily intakes of nitrogen, gains in weight, or nitrogen efficiencies. Growth was excellent, being from 4.8 to 5.3 g. per day. The gain per gram of nitrogen ingested from all sources equalled or exceeded that associated with 1.6% of nitrogen, the maximum value being 22.5 g. No significant difference existed in respect to nitrogen balances of rats offered 2.0% of nitrogen from the 5 rations, when expressed on the basis of either actual values or per 100 sq. cm. of body surface. Correlation analyses, however, indicated that the meat cooked to 234°F. (112.2°C.) was not homogeneous with the others in respect to certain relationships involving nitrogen balance. In contrast to the positive correlation characteristic of the other 4 groups, a negative correlation existed between nitrogen balance, urinary and fecal nitrogen, and surface area. Although the quantity of urinary nitrogen excreted by the animals fed beef cooked to the highest internal temperature was greater ($P < .01$) than that excreted by other groups, fecal nitrogen was not increased significantly. Interestingly, the rats fed beef cooked to an internal temperature of 234°F. (112.2°C.) had significantly larger livers ($P < .05$) with higher concentrations of nitrogen ($P < .01$) than did the other rats. Average total quantities of nitrogen present in the entire carcasses, including the livers, of all groups fed 2.0% of nitrogen were similar.

Examination by correlation analyses of the pooled data obtained from the rats fed 1.6 and 2.0% of nitrogen, respectively, indicated that when tests for homogeneity were made there was a small but definite advantage in including in the analysis the total quantity of nitrogen in the liver, but not the weight of the liver, after nitrogen balance, fecal nitrogen, and surface area were taken into account.

The nutritional superiority of the diets containing 2.0% of nitrogen over those supplying 1.6% is indicated by the fact that the rats fed the higher percentage retained additional nitrogen. During the 28-day growth interval, an increase of approximately 42% in dietary nitrogen was reflected by increments of 36% in the average quantities of nitrogen present in livers and carcasses above those characteristic of rats fed 1.6% of nitrogen. During the 7-day balance period, the average nitrogen intake per 100 sq. cm. of rats fed 2.0% nitrogen was 37.0% higher than that of the other animals, and the corresponding nitrogen balance 44.0% greater. Nevertheless, under the experimental conditions that were imposed, the diet supplying 1.6% of nitrogen from beef was as useful as that providing 2.0% for comparing the nutritive values of beef cooked under various conditions, since no marked overall differences were revealed in either case.

It may be concluded, therefore, that beef may be braised or cooked in a pressure saucepan to internal temperatures of either 176 or 234°F. without a significant decrease in the nutritive value of the proteins, as measured by growth or nitrogen balance in the growing rat. These findings agree with those of other workers *(6, 7, 8)* who used home cooking methods.

SUMMARY

Paired top and bottom round steaks cut from 3 carcasses, graded U. S. Commercial, were cooked by the following methods: *(a)* under 10 p.s.i.g. pressure to an internal temperature of 176°F. (80°C.), *(b)* under 15 p.s.i.g. pressure to an internal temperature of 176°F. (80°C.), *(c)* under 15 p.s.i.g. pressure to an internal temperature of 234°F. (112.2°C.), and *(d)* braised to an internal temperature of 176°F. (80°C.) in a rotary gas oven maintained at 300°F. (148.9°C.).

The internal temperature to which the meat was cooked, rather than the method of cooking, was important in determining the cooking losses and palatability of the steaks. Those cooked to 176°F. (80°C.) lost less weight during cooking, and were less tender, but more desirable in aroma, flavor, and juiciness than steaks cooked to 234°F. (112.2°C.).

None of the cooking methods lowered the nutritive value of the meat when measured in terms of growth and nitrogen retention in the growing rat. Under the conditions of the experiment, a diet supplying 1.6% of nitrogen from beef was as useful as a diet providing 2.0% of nitrogen for comparing the nutritive value of beef prepared by different methods.

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STABILIZING APPLE WINE WITH GLUCOSE OXIDASE[a, b]

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Apple wine is susceptible to oxidation and to the development of acetic acid. Pasteurizing or adding sulfur dioxide has been the general practice in stabilizing apple wine. Pasteurization is practical only when the wine is bottled and hermetically sealed. It sometimes causes a burnt taste in the apple wine. Addition of sulfur dioxide causes an undesirable pungent taste; moreover, the sulfur dioxide loses its effectiveness as a preservative when combined with other compounds. This article introduces another method for stabilizing apple wine; namely, the use of an enzyme preparation.

THE ENZYME SYSTEM

The enzyme used in this experiment, a commercial preparation[c] consisting of glucose oxidase and catalase, catalyzes the oxidation of glucose to gluconic acid by utilizing both glucose and oxygen. It is supplied in two forms, liquid and powder. The powder, having 9 times the strength of the liquid product, was used in this experiment.

Use of this enzyme preparation for removing glucose from egg white has been successfully demonstrated by other investigators *(1)*. Although its optimum pH range has been reported by the manufacturer as 4.5 to 8.0, it has been shown that the enzyme will survive a lower pH and yet be effective *(3)*. According to its manufacturer, the optimum temperature range of the enzyme preparation is 70 to 100°F.

PRELIMINARY EXPERIMENT

Since the apple wine under investigation had a pH of 3.6 and contained 12% of alcohol by volume, it was deemed necessary to conduct a preliminary experiment to determine the activity of the enzyme preparation under such circumstances.

Other investigators *(2)* have determined that the formation of gluconic acid is equivalent quantitatively to the oxygen uptake required for the oxidation of glucose under the enzyme system. In other words, the production of gluconic acid is a quantitative indication of enzyme activity *(4)*. Based on this finding, the activity of the enzyme at pH 3.6 and in the presence of 12% alcohol was determined.

A solution containing 1% glucose and 12% ethyl alcohol was prepared. It was brought to pH 3.6 with malic acid (the acid present in apples). Ten ml. of the solution was titrated to phenolphthalein end-point with 0.01 N. sodium hydroxide, the amount of sodium hydroxide required to neutralize the malic acid. The additional sodium hydroxide used in the subsequent titrations was, then, due to the formation of gluconic acid. The additional quantity of 0.01 N. sodium hydroxide required to titrate the gluconic acid permitted the activity of the enzyme to be determined: the more sodium hydroxide used, the higher the enzyme activity.

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[a] Presented before the 14th Annual Meeting of the Institute of Food Technologists, Los Angeles, California, June 28, 1954.

[b] Approved for publication by the Director of the Oregon Agricultural Experiment Station as Technical Paper No. 872. Contribution of the Department of Food Technology.

[c] DeeO, manufactured by the Takamine Laboratory.

One-tenth of 1% of the enzyme was added to the glucose-alcohol-malic acid solution. The gluconic acid production was determined at subsequent intervals. To determine the effect of enzyme concentration in the solution, 0.15% of the enzyme was also used and the results were compared. For control, a 1% glucose solution without alcohol and malic acid was used. This solution had a pH of 6.0, well within the optimum pH range of 4.5 to 8.0. One-tenth of 1% of the enzyme was added, and the gluconic acid production was determined as before. The experiment was conducted at room temperature.

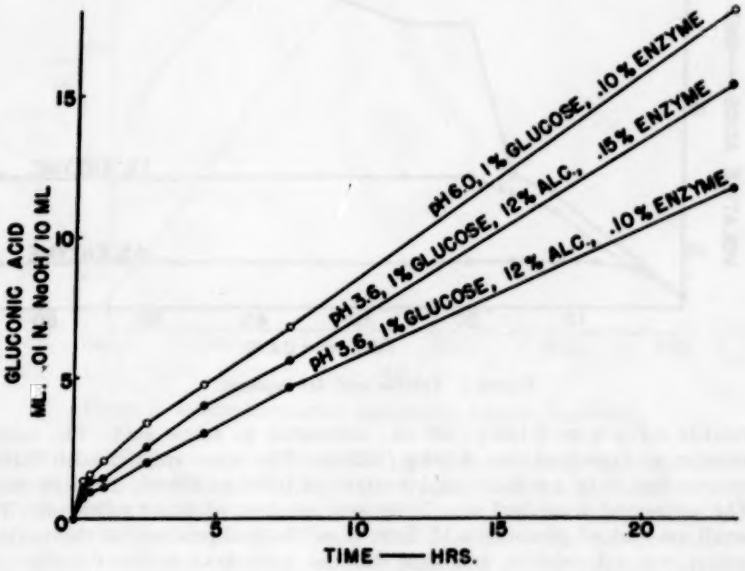

Figure 1. Activity of enzyme.

The results of this preliminary experiment, shown in Figure 1, indicate (a) the enzyme is active at pH 3.6 and in the presence of 12% alcohol; (b) the enzyme is more active at pH 6.0, without alcohol; (c) at pH 3.6 and in 12% alcohol, a concentration of 0.15% of the enzyme has higher activity than 0.10%. By this preliminary experiment it was reasonably ascertained that the enzyme will survive sufficiently to be effective in a medium such as apple wine.

EXPERIMENTAL

Apple wine was made in the usual manner *(5)* except that it was not pasteurized or otherwise stabilized with sulfur dioxide. At the end of fermentation the wine had a pH of 3.6, contained 12% alcohol by volume, and 1.2% glucose. After the fermentation was completed, the wine was divided into 3 lots, one of which was treated with 0.10% of the enzyme, one with 0.15%; the other, untreated, served as the control. The wines were kept in bottles, approximately 90% full. A sufficient number of bottles were filled to permit examination at subsequent intervals. All the experiments were conducted at room temperature. Stability of the wines was measured by volatile acid development.

RESULTS AND DISCUSSION

The results are shown in Figure 2. The development of volatile acids was retarded by an enzyme content of 0.10%. At the end of 64 days, the

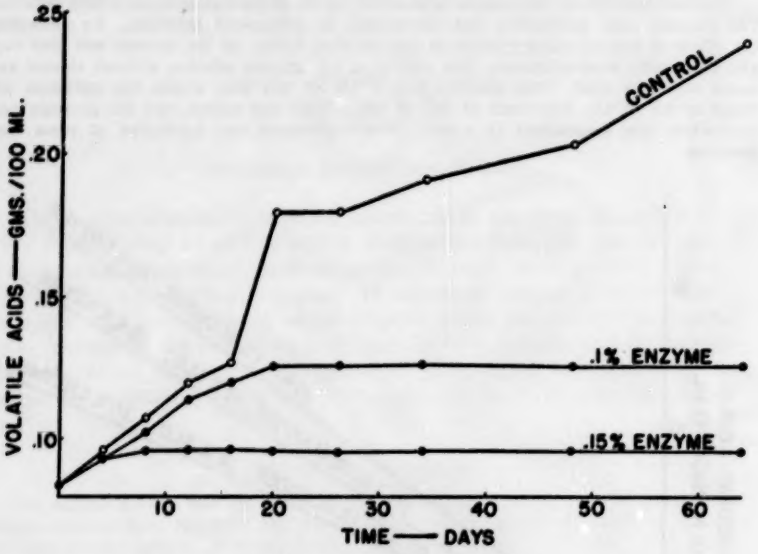

Figure 2. Volatile acid development.

volatile acids were 0.126 g./100 ml., calculated as acetic acid. The initial volatile acid content was 0.084 g./100 ml. The wine treated with 0.15% enzyme had only a volatile acid content of 0.096 g./100 ml. after 64 days. The untreated wine had a volatile acid content of 0.240 g./100 ml. The small amount of gluconic acid formed as the end-product of the enzyme action was not volatile, and thus did not contribute to the volatile acid fraction.

The flavor of the enzyme-treated wine was judged by a panel of five experienced fruit wine makers in the states of Oregon and Washington. Taste tests were conducted one month after the wine was treated with the enzyme, and again 4 months after. No objectionable flavors were detected by any of the tasters. The general reaction of the tasters was that it seemed hard to believe that the apple wine was not treated with SO_2, and that it was not pasteurized.

A slight discoloration (browning) was observed in the wine treated with the enzyme. This was probably due to the build-up of hydrogen peroxide as a result of partial inactivation of the catalase component at the low pH.

The discoloration was quite noticeable at the beginning when the wines were fresh. As the wines aged, natural browning took place. The untreated wine became equally as brown as the wine treated with the enzyme.

The color of the wines was compared both visually and with a Beckman Model DU spectrophotometer. The entire visible spectrum of the wines was observed 2 days after the addition of the enzyme, and again 2 months after.

Figure 3, showing the spectrophotometric characteristics of the apple

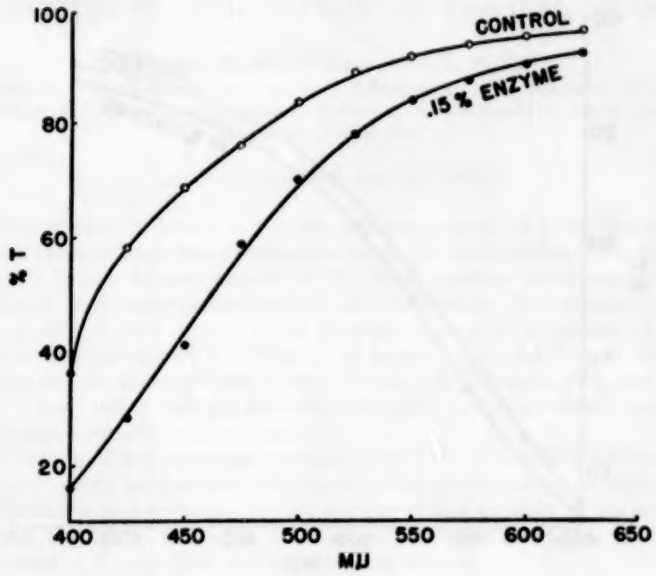

Figure 3. Spectrophotometric characteristics (after two days).

wines 2 days after the enzyme treatment, indicates a considerable difference between the color characteristics of the treated and the untreated wines. The per cent transmission of the enzyme-treated wine was especially low in the blue section of 400 to 475 mμ; this was due to the brown color in the wine. Visual examination likewise revealed the difference between the 2 wines.

The color of the wine was examined again two months after treatment (Figure 4). After two months, natural browning of the wines took place. Both wines were browner, but the untreated wine had caught up with the treated. Although a slight difference in light transmission was still observed by the spectrophotometer, it was not noticeable to the naked eye.

Had the wines been treated with SO_2, a lighter color might have resulted, as the SO_2 has a bleaching effect. Pasteurized wine might also have a lighter color, as pasteurization destroys some enzyme that causes browning. The slight browning caused by the glucose oxidase-catalase enzyme preparation was not considered objectionable by the panel. In fact, a good apple wine should not look too light in color. A little brown adds to the rich appearance of a wine.

SUMMARY

An enzyme preparation of glucose oxidase and catalase has been used to stabilize apple wine by the removal of oxygen and possibly by reducing the growth of acetic acid bacteria. The treated wine was unpasteurized and unsulfured with a pH of 3.6 and an alcohol concentration of 12% by vol-

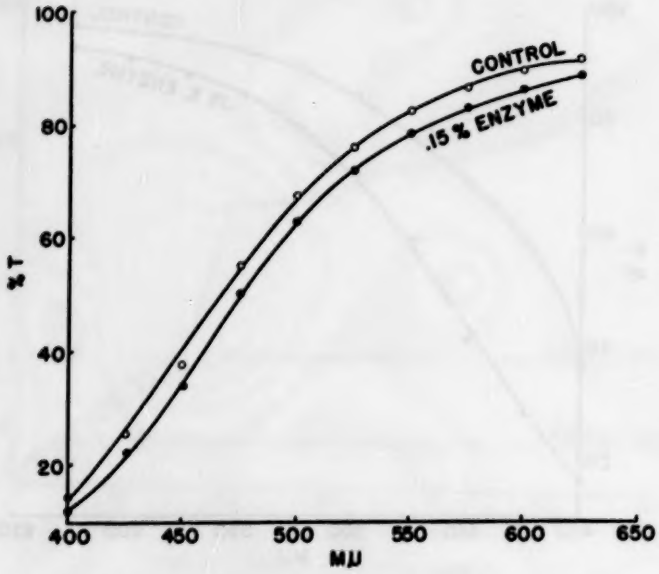

Figure 4. Spectrophotometric characteristics (after two months).

ume. Stability was measured by the development of volatile acid. It was found that the development of volatile acids in the wine was retarded with an enzyme content of 0.10%; further development was stopped by a content of 0.15%. No objectionable flavors were observed, and discoloration was so slight that it showed no appreciable difference from the natural browning of the untreated wine.

Acknowledgment

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PANTOTHENIC ACID CONTENT OF TROPICAL FOODS

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Although little is known about the real significance of pantothenic acid in the human economy, this substance is suspected to be essential for normal nutrition. It is a key-component of the main reaction involving cellular oxidation of food materials in the body, as demonstrated by Lipmann *(6, 7)*, Korkes *et al. (5)*, and others. Until recently no specific symptoms in man have been correlated with a deficiency of pantothenic acid, but in the last few years several investigators, Denny-Brown *(3)*, Glusman *(4)*, and Vernon *(10)*, have noted that the so-called "burning feet syndrome" responds with dramatic results to this vitamin.

As a search of the literature revealed very little up-to-date information on the pantothenic acid content of tropical foods, the following investigation was undertaken to determine the distribution of this vitamin in the cereals, fruits, and vegetables, as well as in a few animal foodstuffs, either produced or consumed in Puerto Rico and neighboring islands.

MATERIAL AND METHODS

The large majority of the foods assayed were purchased at various local markets or from street vendors in the San Juan metropolitan area. A few fruits not available at the markets were obtained directly from the areas in the interior, where they were produced. The majority of the samples used were therefore representative of what average consumers buy. In most cases, at least 3 different samples of each material were assayed. In Table 1 the number of samples assayed as well as the maximum and minimum values obtained and the average pantothenic acid content for the material are recorded.

The assay procedure was that of the Association of Vitamin Chemists *(1)*. Samples were pretreated with chicken liver extract and alkaline intestinal phosphatase for a more complete liberation of bound forms of pantothenic acid, as indicated by Neilands and Strong *(8)*. *Lactobacillus arabinosus* obtained from the American Type Collection in Washington, D. C. was used as test organism. All the plant material has been tabulated with the scientific nomenclature accorded it by Britton and Wilson *(2)*. The common English names used are those recorded by the last named authors or by Otero, Toro, and Otero *(9)*.

RESULTS

Among the fruits found to be high in pantothenic acid the pulp of the avocado (*Persea Persea* L.) was 1,090 μg./100 g., and the pulp and rind of the passionflower fruit (*Passiflora laurifolia* L.) were 1,550 and 1,867 μg./100 g., respectively. Among the legumes, the chick peas (*Cicer arietinum* L.) and the black beans (*Dolichos lablab* L.) showed values of the order of 1,251 and 1,232 μg./100 g., respectively. Among the greens, the wild ripe peppers scored a pantothenic acid content well above that of any other member of this group (1,081 μg./100 g.). Among the seeds assayed, the stinking weed seed (*Ditremexa occidentalis* L.) 2,655 μg./100 g., breadfruit

TABLE 1

Pantothenic acid content of tropical foods

Material			Description of sample	No. of samples tested	Estimated moisture %	Micrograms per 100 grams of edible portion		
Common name in English	Scientific name	Common name in Spanish				Max.	Min.	Ave.
Vegetable products:								
Cereal and cereal products:								
Corn	*Zea mays L.*	**Maíz**	**Fresh from the cob**	**3**	**10.7**	**404**	**260**	**338**
Corn meal	*Zea mays L.*	**Harina de maíz**		**3**	**15.0**	**597**	**427**	**523**
Rice (native)	*Oryza sativa L.*	**Arroz del país**		**1**	**11.8**	.....	.....	**515**
Rice (polished)	*Oryza sativa L.*	**Arroz pulido**	**Imported**	**3**	**12.7**	**589**	**531**	**526**
Oats	*Avena sativa L.*	**Avena**	**Whole oats**	**2**	**10.0**	**1,037**	**1,033**	**1,035**
Fruits:								
Avocado	*Persea Persea L.*	**Aguacate**	**Ripe**	**4**	**77.7**	**1,221**	**921**	**1,090**
Banana, var. apple	*Musa sapientum L.*	**Guineo manzano**	**Ripe**	**3**	**71.8**	**430**	**202**	**296**
Banana, var. fig.	*Musa sapientum L.*	**Guineo niño**	**Ripe**	**3**	**71.8**	**235**	**160**	**194**
Banana var. montecristo	*Musa sapientum L.*	**Guineo montecristo**	**Ripe**	**1**	**71.8**	.....	.....	**136**
Breadfruit	*Artocarpus communis Forst*	**Panapén**	**Ripe**	**3**	**64.9**	**529**	**417**	**457**
Cashew fruit	*Anacardium occidentale L.*	**Pajuil**	**Ripe**	**3**	**86.3**	**102**	**55**	**76**
Coco plum	*Chrysobalanus icaco L.*	**Hicaco**	**Ripe**	**3**	**86.1**	**83**	**71**	**77**
Custard apple	*Annona reticulata L.*	**Corazón**	**Ripe**	**6**	**73.1**	**156**	**89**	**135**
Genipa	*Genipa americana L.*	**Jagua**	**Ripe, pulp**	**6**	**80.1**	**126**	**27**	**55**
Golden apple	*Spondias dulcis Frost*	**Jobo de la India**	**Green**	**1**	.....	.....	.....	**221**
Golden apple	*Spondias dulcis Frost*	**Jobo de la India**	**Ripe**	**4**	**85.1**	**286**	**190**	**259**
Grapefruit	*Citrus maxima Burm.*	**Toronja**	**Juice**	**4**	**81.1**	**201**	**89**	**156**
Guava (red pulp)	*Psidium guajava L.*	**Guayaba**	**Whole edible part (pulp and rind)**	**3**	**81.1**	**213**	**119**	**166**
Guava (white pulp)	*Psidium guajava L.*	**Guayaba**	**Whole edible part (pulp and rind)**	**3**	.....	**154**	**110**	**127**
Kanapy, bullace plum	*Melicocca bijuga L.*	**Quenepa**	**Ripe**	**4**	**76.7**	**319**	**150**	**221**
Lemon	*Citrus limonia Osbeck*	**Limón**	**Juice and pulp**	**4**	**85.8**	**134**	**71**	**108**
Lime, sweet	*Citrus limetta Risso*	**Limón dulce**	**Juice**	**4**	**90.3**	**334**	**286**	**314**

Mamey	*Mammea americana*	Mamey	Ripe	5	86.2	129	71	103
Mango	*Mangifera indica L.*	Mangó	Ripe	3	83.6	178	128	160
Mesple	*Sapote achras Mill*	Níspero	Ripe	3	76.4	272	227	252
Orange	*Citrus sinensis L.*	China	Juice and pulp	5	87.2	229	132	165
Orange, sour	*Citrus aurantium L.*	Naranja	Juice and pulp	3	82.7	241	215	232
"Papaya"	*Carica papaya L.*	Papaya	Ripe	4	89.6	321	129	218
Passionflower fruit	*Passiflora laurifolia L.*	Parcha	Pulp and juice	4	79.5	1,712	1,245	1,550
Passionflower fruit	*Passiflora laurifolia L.*	Parcha	Rind	4	.....	2,193	1,420	1,867
"Pepino angolo"	*Sicana odorifera Vell.*	Pepino angolo	Ripe	4	87.2	280	119	232
Pineapple	*Ananas Ananas L.*	Piña	Ripe	4	85.4	194	61	147
Pomegranate	*Punica granatum L.*	Granada	Pulp covering seeds	3	79.4	605	596	542
Pomegranate	*Punica granatum L.*	Granada	Rind	2	.....	232	171	202
Soursop	*Annona muricata L.*	Guanábana	Juice and pulp	3	81.2	269	225	253
Soursop	*Annona muricata L.*	Guanábana	Juice	2	.....	292	278	285
Spanish plum	*Spondias purpurea L.*	Jobillo	Whole edible part	3	76.7	322	265	296
Star apple	*Chrysophylum cainito L.*	Caimito	Whole edible part	3	82.8	522	372	444
Sweetsop	*Annona squamosa L.*	Anón	Pulp, ripe	3	64.6	243	206	226
Tamarind	*Tamarindus indica L.*	Tamarindo	Pulp surrounding seeds	4	37.7	328	80	143
West Indian cherry	*Malpighia punicifolia L.*	Acerola	Juice	3	86.3	247	153	205
West Indian locust	*Hymenoea courbaril L.*	Algarrobo	Yellow powder surrounding seeds	3	14.6	606	437	520
Vegetables:								
Root crops:								
Arracacha	*Arracacia Xanthorrhiza Bancroft*	Apio	As sold in the market	3	74.8	1,008	232	583
Carrot	*Daucus carota L.*	Zanahoria	As sold in the market	4	88.2	363	251	315
Carrot	*Daucus carota L.*	Zanahoria	As sold in the market, cooked	1	.....	.....	.....	504
Cassava	*Manihot Manihot L.*	Yuca	As sold in the market	3	56.2	656	320	521
Corn root, sweet	*Calathea allouia Aubl.*	Lerenes	As sold in the market	2	83.2	510	369	440
"Maguey" root	*Furcraea tuberosa Ait. f.*	Raíz de maguey	As sold in the market	1	.....	.....	.....	592

TABLE 1 (continued)

Material			Description of sample	No. of samples tested	Estimated moisture %	Micrograms per 100 grams of edible portion		
Common name in English	Scientific name	Common name in Spanish				Max.	Min.	Ave.
Nut-grass	*Cyperus rotundus L.*	Coquí	As sold in the market	2	48.7	539	495	517
Potato	*Solanum tuberosum L.*	Papa	As sold in the market	3	78.9	418	116	267
Puerto Rican sweet potato	*Ipomoea batatas L.*	Batata mameya	As sold in the market	5	70.0	693	425	513
Sweet potato	*Ipomoea batatas L.*	Batata	As sold in the market	3	69.0	814	422	605
Sweet potato	*Ipomoea batatas L.*	Batata	As sold in the market, cooked	1	.....	.....	.....	526
Yam, white	*Dioscorea alata L.*	Ñame blanco	As sold in the market	3	69.3	838	472	629
Yam, yellow	*Dioscorea alata L.*	Ñame amarillo	As sold in the market	1	72.0	.....	.....	386
Yautía, white	*Xanthosoma sagittifolium L.*	Yautía blanca	As sold in the market	4	67.5	1,032	580	719
Yautía, yellow	*Xanthosoma sagittifolium L.*	Yautía amarilla	As sold in the market	3	61.4	1,121	629	895
Beans:								
Beans ("galanas")		Habichuelas galanas	Dried	1	.....	.....	.....	661
Black beans	*Dolichos lablab L.*	Chícharos	Dried	4	11.6	1,465	979	1,232
Cowpea	*Vigna unguiculata L.*	Frijoles (grandes)	Dried	4	14.8	1,332	549	900
Cowpea	*Vigna unguiculata L.*	Frijoles (pequeños)	Dried	2	.....	1,070	648	859
Cowpea	*Vigna unguiculata L.*	Frijoles (del país)	Dried, native	2	.....	1,116	391	754
Cowpea	*Vigna unguiculata L.*	Frijoles (americanos de California)	Dried, imported	3	.....	1,320	1,082	1,174
Cuban black beans		Hab. negras de Cuba	Dried, imported	1	.....	.....	.....	516
Chick pea	*Cicer arietinum L.*	Garbanzos	Dried, imported	4	17.3	2,092	879	1,251
Lima beans	*Phaseolus lunatus L.*	Habas americanas	Dried, imported	3	10.4	1,005	693	812
Lima beans	*Phaseolus lunatus L.*	Habas (grandes de California)	Dried, imported	1	.....	.....	.....	996

Lima beans	*Phaseolus lunatus L.*	Habas (pequeñas de California)	Dried, imported	3	.....	814	640	727
Lima beans	*Phaseolus lunatus L.*	Habas (pintas de California)	Dried, imported	1	.....	.....	.....	658
Navy beans	*Phaseolus vulgaris L.*	Hab. blancas americanas	Dried, imported	3	.....	463	362	423
Navy beans	*Phaseolus vulgaris L.*	Hab. blancas del país	Dried, native	4	12.6	724	319	573
Pigeon pea	*Cajanus cajan L.*	Gandules del país	Fresh, native	4	62.4	937	577	680
Red kidney beans	*Phaseolus vulgaris L.*	Hab. coloradas del país	Dried, native	1	.....	.....	.....	225
Red kidney beans	*Phaseolus vulgaris L.*	Hab. coloradas americanas	Imported (cooked & dried)	3	15.0	629	356	512
Rose beans	*Phaseolus vulgaris L.*	Hab. rositas	Dried	3	16.0	710	629	682
Spanish lentils	*Ervum Lens L.*	Lentejas españolas	Dried, imported	1	15.0	.....	.....	1,144
String beans	*Phaseolus vulgaris L.*	Hab. tiernas	Fresh, native	4	89.2	327	163	214
Greens:								
Lettuce	*Lactuca sativa L.*	Lechuga del país	Fresh, native	3	94.7	159	121	142
Lettuce	*Lactuca sativa L.*	Lechuga americana	Fresh imported	1	95.0	.....	.....	129
Okra	*Abelmoschus esculentus L.*	Quingombó	Fresh	4	90.2	356	179	286
Peppers, green	*Capsicum frutescens L.*	Pimiento verde	Fresh	3	92.4	190	187	188
Peppers, sweet	*Capsicum frutescens L.*	Ají dulce verde	Fresh, green	3	89.2	301	162	227
Peppers, sweet	*Capsicum frutescens L.*	Ají dulce maduro	Fresh, ripe	3	90.0	298	226	271
Peppers, wild	*Capsicum frutescens L.*	Ají bravo verde	Fresh, green	3	90.0	1,046	479	687
Peppers, wild	*Capsicum frutescens L.*	Ají bravo maduro	Fresh, ripe	3	91.0	1,241	886	1,081
Cabbage	*Brassica oleracea L.*	Repollo	Fresh	3	91.5	175	153	163
Watercress	*Roripa nasturtium aquaticum L.*	Berros	Fresh	3	93.6	244	154	210
Seeds:								
Almond, tropical	*Terminalia catappa L.*	Almendra tropical	Kernel	3	9.3	459	363	409

TABLE 1 (concluded)

Material			Description of sample	No. of samples tested	Estimated moisture %	Micrograms per 100 grams of edible portion		
Common name in English	Scientific name	Common name in Spanish				Max.	Min.	Ave.
Breadfruit seed	*Artocarpus communis Forst.*	Pepita de pan	Edible part	4	64.0	2,430	1,109	1,715
Cashew nut	*Anacardium occidentale L.*	Nuez del pajuil	Kernel	6	5.3	1,700	1,225	1,488
Coconut	*Cocos nucifera L.*	Coco seco	Flesh from dried coconut	3	60.7	213	198	207
Coconut	*Cocos nucifera L.*	Agua de coco	Water from dried coconut	2	94.6	0.52	0.51	0.52
Coffee senna (stinking weed)	*Ditremeza occidentalis L.*	Hedionda	Whole seed	1	.....	.....	.....	2,655
Sesame	*Sesamum orientale L.*	Ajonjolí	Whole seed	2	5.6	437	319	378
Other types:								
Calabash	*Crescentia cujete L.*	Higüera	Ripe, pulp	3	40	170	136	158
"Chayote"	*Sechium edule Jacq.*	Chayote	Ripe	3	89.8	545	366	483
Egg plant	*Solanum melongena L.*	Berenjena	Ripe	3	90.8	284	195	229
Onion	*Allium cepa L.*	Cebolla	Whole	3	87.6	165	105	127
Plantain, green	*Musa paradisiaca L.*	Plátano verde	As sold in the market	3	66.8	653	223	372
Plantain, green	*Musa paradisiaca L.*	Tostones	Fried plantain	1	.....	.....	.....	334
Plantain, yellow	*Musa paradisiaca L.*	Plátano amarillo	As sold in the market	4	67.5	353	112	247
Squash	*Cucurbita moschata Duch*	Calabaza	Ripe	4	91.1	373	228	306
Tomato	*Lycopersicon Lycopersicon L.*	Tomate	Ripe, imported	3	94.3	130	54	92
Animal products:								
Dairy products:								
Chocolate ice cream		Mantecado de chocolate	As sold	1	62.0	.....	.....	331

Vanilla ice cream		Mantecado de vainilla	As sold	1	62.0	.....	.....	492
Cheese (Puerto Rican)		Queso blanco del país	Resembles cottage cheese	3	53.0	284	149	198
Milk (cow's)		Leche de vaca	Pasteurized and homogenized	3	87.0	374	324	343
Eggs:								
Hen's egg (white)		Clara de huevo	As sold in the market	3	87.7	353	208	287
Hen's egg (yolk)		Yema de huevo	As sold in the market	4	49.4	4,624	1,848	3,121
Fish:								
Dried salted codfish		Bacalao	Imported	3	about 20	1,077	753	905
Clam		Almeja	Fresh	3	80.0	723	518	593
Shrimp		Camarones del río	Boiled in salted water	3	.....	247	179	214
Lobster		Langosta cocida	Cooked	2	.....	440	269	355
Miscellaneous:								
Guava paste	*Psidium guajava L.*	Pasta de guayaba	As sold in the market	3	25.0	228	187	205
Ginger	*Zingiber Zingiber L.*	Jengibre	As sold in the market	4	26.0	291	118	203
Honey (bee's)		Miel de abeja	Local product	3	34.0	281	203	229
Mixture of 2/3 rice, 1/3 red kidney beans		Mezcla de 2/3 arroz, 1/3 hab. coloradas	Cooked, dried and ground mixture	1	6.0	.....	.....	463
Molasses, blackstrap		Miel de purga	Local product	1	24.0	.....	.....	492
Molasses, edible		Melao	Concentrated cane sugar juice	3	24.0	643	110	462
Yeast (brewers type) 2019		Levadura		3	6.0	17,000	14,389	15,911
Yeast *(Torula utilis)*		Levadura	Manufactured at the Pilot Plant of "Cía. de Fomento de P. R."	3	5.4	11,061	5,506	9,002

seed (*Artocarpus communis* Forst) 1,715 μg./100 g., and cashew nut (*Anacardium occidentale* L.) 1,488 μg./100 g. had the highest content. Food yeast (*Torula utilis*) had a pantothenic acid content of 9,002 μg./100 g., while brewers yeast had nearly twice as much. Among the few animal products assayed, the hen's egg yolk was the highest, containing 3,121 μg./100 g. The egg white, on the other hand, was relatively low, containing only 287μg./-100 g.

SUMMARY

The pantothenic acid content of 123 different foodstuffs produced or consumed in Puerto Rico and neighboring islands has been determined by the microbiological assay method.

Acknowledgment

We want to thank Dr. Frank M. Strong of the Department of Biochemistry of the University of Wisconsin for providing a sample of liver concentrate of known pantothenic acid content against which our enzymes preparations were routinarily checked.

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DISTRIBUTION OF CAROTENOIDS IN THE TOMATO

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MacGillivray *(2, 3)* divided tomato fruits into regions, which he found to vary not only in total and soluble solids but in color as well. Puree from the outer and inner wall was of a redder hue than that from the locular jelly. He also found that the per cent of outer wall and locule pulp varied with variety and season. Younkin *(7)* also showed that puree from the outer wall had the best and that from the locular contents the poorest color. Ellis and Hamner *(1)* found that the carotene content was higher in the stem than the flower end of the tomato and therefore used a median (transverse) slice in sampling fruits. The exposed and shaded sides of a tomato have been shown to vary in carotenoids due to differences in temperature and illumination *(5)*.

When less than the whole fruit is sampled for carotenoid determinations, it is important to know just how these pigments are distributed in the fruit and the factors that affect their distribution. Also of interest is the relation of the color of the regions of the fruit to the carotenoid content.

METHODS

Except where otherwise noted samples consisted of 12-15 fruits selected for uniformity. When a reddish tint was first apparent at the apical end of the fruit, it was considered to be at the turning stage. Fruits were divided into regions by removing the locular contents from sectors of a fruit and separating the outer and inner pericarp. Each fraction of the sample was weighed, blended for 2 minutes in a Waring blender and strained through doubled cheese cloth to remove the skins and seeds. Total carotenoids and carotene were determined by a method previously described *(4)*. Color readings were made with a Hunter color difference meter and recorded as a/b ratio.

EXPERIMENTAL RESULTS

Fruits of the Garden State, Stokesdale, and Rutgers varieties grown in the field and those of the Lloyd Forcing variety grown in the greenhouse were harvested at the turning stage and ripened for 12 days at 20°C. The fruits of each variety were separated into outer pericarp, inner pericarp, and locular contents and analyzed for total carotenoids and carotene. The data (Table 1) show a similar relationship between the carotenoids in all varieties. The outer pericarp was much higher in total carotenoids than the internal parts of the fruit. Locular contents were similar to the inner pericarp in total carotenoids but 3 to 4 times as high in carotene, which resulted in a low total carotenoid-carotene ratio and undesired color.

To determine the effect of ripening on the carotenoids in the various parts of the tomato the Stokesdale variety was grown as a spring crop in the greenhouse. Turning fruits were harvested at 4-day intervals and ripened at 20°C. Samples ripened respectively for 4, 8, 12, 16, and 20 days were separated into outer pericarp, inner pericarp, and locular contents and analyzed for total carotenoids, carotene, and color. The data (Table 2) show that except for the 4-day fruits total carotenoids were highest in the outer wall and the locular contents were highest in carotene. The a/b (color) readings increased with total carotenoids and decreased with carotene. The difference between external and internal color persisted until the fruits were soft ripe.

The effect of season on the relation of internal to external color of the fruit was investigated with the Early Baltimore variety grown at Des Plaines, Illinois. Turning

TABLE 1

Carotenoids in the different regions of the fruit

Region of fruit	Per cent of total	Carotenoids (mg./100 g.)		
		Total	Carotene	Ratio
Garden State				
Outer pericarp	42.9	13.84	0.53	26
Inner pericarp	38.3	8.12	0.27	30
Locular contents	18.8	9.16	1.02	9
Stokesdale				
Outer pericarp	53.9	13.20	0.43	31
Inner pericarp	28.8	7.20	0.38	19
Locular contents	17.3	8.16	1.68	5
Rutgers				
Outer pericarp	47.0	10.20	0.50	25
Inner pericarp	28.6	6.24	0.20	31
Locular contents	24.4	5.28	1.02	5
Lloyd Forcing				
Outer pericarp	54.3	6.60	0.38	18
Inner pericarp	25.3	3.52	0.30	12
Locular contents	20.4	3.12	0.82	4

and U. S. No. 2 fruits (based on external color) were harvested August 30 and September 9, 16, and 22. Each sample was brought to Urbana and analyzed for total carotenoids on the day following harvest. The data (Table 3) show that total carotenoids increased with successive harvests, indicating greater internal as compared with external color development as the season advanced.

Ripe shaded fruits of Garden State, a canning variety, University of Illinois, Accession 1075 with 2 loculed fruits and Webb Special, high in carotenoids, were har-

TABLE 2

Carotenoids and color of the regions of the tomato[1] at different stages of ripeness

Region of fruit	Number of days ripened				
	4	8	12	16	20
Total carotenoids (mg./100 g.)					
Outer pericarp	4.40	7.84	8.64	8.64	8.40
Inner pericarp	2.96	4.40	4.40	4.00	4.40
Locular contents	4.80	4.16	4.00	6.56	6.32
Carotene (mg./100 g.)					
Outer pericarp	.23	.23	.18	.18	.26
Inner pericarp	.22	.12	.15	.18	.39
Locular contents	.69	.71	.60	.57	.55
Total carotenoids/carotene					
Outer pericarp	15	34	47	47	23
Inner pericarp	14	37	30	22	11
Locular contents	7	6	7	12	12
Color (a/b)					
Outer pericarp	1.71	2.44	2.60	2.52	2.47
Inner pericarp	1.75	2.15	2.04	1.99	1.80
Locular contents	1.34	1.56	1.59	1.59	1.71

[1] Variety—Stokesdale.

TABLE 3

Effect of season on total carotenoids of turning and No. 2 fruits[1] (mg./100 g.)

Stage of ripeness	Date of harvest			
	August 30	September 9	September 16	September 22
Turning	2.04	2.74	3.00	4.20
U.S. No. 2	4.46	5.40	6.56	9.20

[1] Variety: Early Baltimore.

vested and separated into pericarp and locular contents. Each was weighed and analyzed for total carotenoids and carotene. The data (Table 4) show that Garden State and Webb Special were similar in per cent locular content but the latter was higher in both total carotenoids and carotene. Accession 1075 was about 3 times as high as the other varieties in per cent locular content, which was similar in carotenoids to that of Garden State. Although the carotene content of the pericarp of Accession 1075 was lower than that of Garden State, the carotene content of the whole fruit was higher because of its high per cent of locular tissue.

The effect of exposure to light on the development of carotenoids in the apical and basal ends of the fruit was tested with Garden State and Illinois T 19. Three samples of ripe fruit with apical end, basal end, and cheek, respectively, exposed to sunlight were used. The fruits were quartered and the outer pericarp removed and cut into apical and basal halves. These were analyzed separately for total carotenoids and carotene. The data (Table 5) show that when the apical ends were exposed to sunlight total carotenoids and carotene in both ends were similar. When the basal ends or cheeks were exposed, the pericarp toward the apical end was higher in total carotenoids but lower in carotene.

Since carotene is always associated with chlorophyll, a number of tests were made to determine if the higher carotene toward the basal end of green-shouldered fruits would also be found in those with the uniform color gene. Several samples of both types of fruit with central axes parallel to the ground were harvested. The outer pericarp from opposite sectors was divided into apical and basal halves and analyzed separately for total carotenoids and carotene. The data (Table 6) show that in both types of fruits the apical pericarp was higher in total carotenoids. The basal ends were higher in carotene, but the difference in the fruits with the uniform color gene was small and not significant.

The effect of ripeness on the carotene in the apical and basal pericarp was determined with the Illinois T 19 variety. Two samples differing in degree of ripeness were selected and analyzed as in the above experiment. In the partially ripened sample (Table 7) the pericarp of the apical end was higher than that of the basal end of the fruit in total carotenoids; there was no difference in carotene. In the fully ripe fruits both ends had reached the same content of total carotenoids, but the basal end was highest in carotene.

TABLE 4

Carotenoids in relation to locular content

Variety	Part of fruit	Per cent	Carotenoids (mg./100 g.)	
			Total	Carotene
Garden State	Pericarp	86.47	10.28	0.41
	Locular content	13.53	7.72	0.97
	Fruit ave.		9.93	0.48
U. of I. Accession 1075	Pericarp	59.04	13.36	0.34
	Locular content	40.96	8.20	1.02
	Fruit ave.		11.25	0.62
Webb special	Pericarp	86.03	17.32	0.84
	Locular content	13.97	8.72	1.41
	Fruit ave.		16.12	0.92

TABLE 5

Carotenoid content of apical and basal pericarp of the tomato in relation to sunlight

Exposure	Part analyzed	Garden State Carotenoids (mg./100 g.)		Illinois T 19 Carotenoids (mg./100 g.)	
		Total	Carotene	Total	Carotene
Apical	Basal	7.76	.536	10.96	.960
	Apical	7.92	.552	10.08	.800
Basal	Basal	5.28	.680	5.76	.720
	Apical	9.00	.472	9.04	.512
Cheek	Basal	6.76	.664	9.36	.752
	Apical	7.40	.592	10.72	.512

DISCUSSION

Data in Tables 1 and 2 are in agreement with those of other investigators *(4, 7)*, showing that the best color is obtained from the outer pericarp and the poorest from the locular contents. The former was found to be highest in total carotenoids and the latter highest in carotene. The data indicate that chromaticity of tomato products depends upon both total carotenoids and the ratio of lycopene to carotene. Although the content of carotene in the whole tomato amounts to only 2 to 10% of the total carotenoids, it exerts a pronounced effect upon color.

Differences between the external and internal color of the fruit persisted during ripening. They were less in the over-ripe fruits, as was observed by MacGillivray *(3)*. His observations were made early in the season, when the development of internal color is retarded as indicated by the data in Table 3. They also indicate that if the apparent turning stage is used as a criterion for maturity, adjustments must be made during the harvesting season.

Robinson *et al. (6)* showed a decrease in the ratio of juice to external fruit color toward the end of the season in apparent contradiction to the data in Table 3. However, their results were obtained with ripe fruit which often show external yellowing early in the season due to high temperatures and light intensities. These respectively decrease lycopene and increase carotene in the more exposed surface *(5)*.

Data in Table 4 show that varieties may vary in amount of locular material, which is disproportionately high in carotene. Best color should

TABLE 6

Carotenoids of the apical and basal pericarp of fruits with green shoulders and the uniform color gene

Number of tests	Total carotenoids (mg./100 g.)			Carotene (mg./100 g.)		
	Apical	Basal	Difference	Apical	Basal	Difference
Fruits with green shoulders						
8	10.67	11.97	1.30**	.845	.577	.268**
Fruits with uniform color gene						
6	8.26	9.95	1.69*	.585	.507	.078

** Significant at 1% level.
* Significant at 5% level.

TABLE 7

Carotenoid content of the apical and basal pericarp of fruits[1] exposed to light with central axes parallel to ground

Stage of ripeness	Part of pericarp analyzed	Total carotenoids (mg./100 g.)	carotene (mg./100 g.)
Partially ripened	Basal	5.20	.572
	Apical	8.72	.572
Ripe	Basal	10.42	.872
	Apical	10.42	.680

[1] Illinois T 19.

therefore result from high total carotenoids and a low locular content, which reduces the amount of carotene.

Because of its weight and point of attachment a tomato fruit is most likely to have its basal end most exposed to light, which has been found to increase carotene and at low temperatures, lycopene *(5)*. Carotene is also higher in the basal end of ripe fruit because of its association with the green shoulders. Carotenoid development begins in the apical end, where carotene may be higher in the early ripening stage but not in fully colored fruits.

When fruits are sampled for carotenoids or color, equal opposite sectors are recommended. This sampling compensates for unequal exposures to light and for polar and morphological differences.

SUMMARY

The outer pericarp was highest in total carotenoids and the locular contents highest in carotene. The two regions of the fruit resulted respectively in the best and poorest color. Polar variations in the carotenoids were found to be associated with illumination, ripeness, and green shoulders. The normal distribution of carotenoids in the tomato may be altered by exposure to light. Equal opposite sectors of the fruit are recommended in sampling for the carotenoids or color.

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RESISTANCE OF *CLOSTRIDIUM PARABOTULINUM* TO SORBIC ACID[a,b]

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The fungistatic properties of sorbic acid, first reported by Gooding *(5)*, have been verified by a number of investigators. Unfortunately, as already shown by Phillips and Mundt *(12)*, Vaughn and his students *(4, 17, 18)*, and Lindberg, Mason, and Cutchins *(7)*, sorbic acid exerts a selective inhibition, being much more effective against all types of catalase-positive microorganisms tested than against the lactic acid bacteria and clostridia.

Recently, sorbic acid has been recommended as an aid in prevention of molding of wrapped cheese *(2, 3, 8, 9, 10, 11, 13, 14)*. It was thought this compound might also be useful in control of the microbial contamination of dehydrated vegetables. However, since sorbic acid media had already been suggested for isolation of the clostridia *(7, 18)*, it seemed desirable to investigate in more detail the resistance of *C. parabotulinum* and *C. botulinum* types toward this fungistatic compound.

EXPERIMENTAL METHODS AND RESULTS

Forty-two cultures were tested. These included 18 isolates of *C. parabotulinum* type A; 16 isolates of *C. parabotulinum* type B; 5 non-toxic strains of *C. parabotulinum* (6,16); and 1 culture of each of the three types (C, D, and E) of *C. botulinum*. The *C. parabotulinum* isolates were obtained from the collection of the National Canners' Association. The *C. botulinum* cultures were from the collection of Dr. K. F. Meyer.

The 3 basal media used in this study were beef liver infusion; 0.5% Bacto-tryptone broth; and 0.5% Bacto-tryptone, 0.1% Bacto-yeast extract broth. The liver infusion was prepared according to the method of the Committee on Bacteriological Technic *(1)* but *without added phosphate*.

Both vegetative cell and spore suspensions of all cultures were tested for their resistance to sorbic acid. Predominately vegetative cell suspensions were prepared by growing the cultures in plain liver infusion up to about 72 hours, depending upon the activity of the individual isolates. An inoculum always consisted of 0.2 ml. of plain liver infusion culture.

Spore suspensions were prepared by growing the cultures of the different *C. parabotulinum* types in liver infusion plus 0.1% soluble starch and the *C. botulinum* strains in liver infusion containing 0.5 to 1.0 g. of sterile soil until microscopic examination showed a maximum crop of spores had developed. One ml. of the sporulated culture was then added to 9.0 ml. of sterile distilled water, mixed, and heated: the *C. parabotulinum* strains at 100°C. (212°F.) for 10 minutes; and the *C. botulinum* isolates at

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[a] Presented at the Fourteenth Annual Meeting of IFT, Los Angeles, California, June 29, 1954.

[b] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and has been assigned number 498 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

TABLE 1

Effect of concentration of sorbic acid on growth of C. parabotulinum and C. botulinum cultures

Species	Number of strains	Concentration of sorbic acid[1]								
		0	0.12	0.5	1.0	2.0	2.5	3.0	3.5	4.0
		Number growing in 7 days at 35°C. (95°F.)								
Vegetative cell inocula										
C. parabotulinum, type A	18	18	18	18	18	18	18	18	7	0
C. parabotulinum, type B	16	16	16	16	16	16	16	16	4	0
C. botulinum, type C	1	1	1	1	0	0	0	0	0	0
C. botulinum, type D	1	1	1	1	0	0	0	0	0	0
C. botulinum, type E	1	1	1	1	0	0	0	0	0	0
C. parabotulinum, non-toxic[2]	5	5	5	5	5	5	5	0	0	0
Detoxified, washed spore inocula										
C. parabotulinum, type A	18	18	18	18	18	18	14	13	4	0
C. parabotulinum, type B	16	16	16	16	16	16	12	10	2	0
C. botulinum, type C	1	1	1	1	0	0	0	0	0	0
C. botulinum, type D	1	1	1	1	0	0	0	0	0	0
C. botulinum, type E	1	1	1	1	0	0	0	0	0	0
C. parabotulinum, non-toxic[2]	5	5	5	5	5	5	3	3	0	0

[1] Per cent (grams per 100 ml. in liver infusion). Reaction adjusted so pH after sterilization was 6.7 to 6.8. At this pH sodium sorbate is formed.
[2] Non-toxic strains described by Gunnison and Meyer *(6)* and Townsend *(16)*.

TABLE 2

Effect of pH on germination of spores of C. parabotulinum and C. botulinum cultures in 0.12 per cent sorbic acid liver infusion

Species	Number of strains	pH of medium[1]								
		6.0	5.8	5.6	5.4	5.2	5.0	4.9	4.8	4.7	4.6
		Number growing in 7 days at 35°C. (95°F.)									
Medium containing 0.12 per cent sorbic acid											
C. parabotulinum, type A	18	18	18	18	16	14	13	8	5	1	0
C. parabotulinum, type B	16	16	16	16	14	14	10	6	3	0	0
C. botulinum, type C	1	1	1	1	1	0	0	0	0	0	0
C. botulinum, type D	1	1	1	1	1	0	0	0	0	0	0
C. botulinum, type E	1	1	1	1	1	0	0	0	0	0	0
C. parabotulinum, non-toxic[2]	5	5	5	4	4	4	3	0	0	0	0
Medium without sorbic acid											
C. parabotulinum, type A	18	18	18	18	16	14	13	8	5	1	0
C. parabotulinum, type B	16	16	16	16	14	14	10	6	3	0	0
C. botulinum, type C	1	1	1	1	1	0	0	0	0	0	0
C. botulinum, type D	1	1	1	1	1	0	0	0	0	0	0
C. botulinum, type E	1	1	1	1	1	0	0	0	0	0	0
C. parabotulinum, non-toxic[2]	5	5	5	4	4	4	3	0	0	0	0

[1] After sterilization. At approximately pH 5.5 and below, the free sorbic acid is present in the medium.
[2] Non-toxic strains described by Gunnison and Meyer *(6)* and Townsend *(16)*.

80°C. (176°F.) for 5 minutes. An inoculum always consisted of 1.0 ml. of the diluted, heated culture.

The pH values were obtained with the glass electrode. All necessary adjustments were made with *N* NaOH and *N* HCl.

The sorbic acid used in these investigations was purchased from Eastman Kodak Company. An additional sample, donated by Carbide and Carbon Chemicals Co., was also tested for control purposes.

The effect of concentration of sorbic acid. In 1951 Vaughn and Emard *(17)* reported that clostridia could grow in the presence of 0.12% sorbic acid contained in liver infusion. The 10 cultures they investigated represented but three saccharolytic types of the *C. butyricum* complex. More recently York and Vaughn *(18)* have shown that the ability to grow in the presence of sorbic acid in a suitable medium is widespread among the different species of *Clostridium*. It was desirable to test the resistance of a significant number of isolates of *C. parabotulinum* and *C. botulinum* to sorbic acid.

Concentrations of 0.12, 0.5, 1.0, 2.0, 2.5, 3.0, 3.5, and 4.0% sorbic acid were added to plain liver infusion (no K_2HPO_4). Each portion then was adjusted so that the pH value after sterilization was 6.7 to 6.8. Inoculations were made with both vegetative cell and spore suspensions. The test media and controls were incubated for 7 days at 35°C. (95°F.). Development of turbidity and gas were used as criteria for growth.

Considerable difference in resistance to sorbic acid was observed between the isolates of *C. parabotulinum* and *C. botulinum*. At the higher levels of sorbic acid, the tolerance of the *C. parabotulinum* types was notable. There was only slightly more tolerance when vegetative cells were used as the inoculum as shown in Table 1. Regardless of the type of inoculum, all of the cultures grew in the presence of 0.5% sorbic acid.

The effect of pH on germination of spores in the presence of 0.12% sorbic acid. Emard and Vaughn *(4)* showed that pH values in the range of 5.0 to 5.5 improved the selectivity of the 0.12% sorbic acid medium for growth of lactic acid bacteria and suppression of catalase positive microorganisms. The species of *Clostridium* are known to grow better at higher pH values. Therefore, it was desirable to test the effect of pH in germination of the spores of the various botulism cultures.

The effect of pH on germination and growth of spores in the presence of 0.12% sorbic acid was determined by comparison with germination and growth of an aliquot of the same spore suspension in plain liver infusion adjusted to the same pH value.

The limiting pH in the acid range for the different isolates of the 2 species was not appreciably influenced by the presence of 0.12% sorbic acid. Although the ability to germinate and grow in 7 days at 35°C. (95°F.) was decreased markedly as the pH of the medium was reduced from 5.8 to 5.0, a number of isolates of *C. parabotulinum* grew at pH values of 5.0, 4.9, and 4.8, respectively, both in the presence and absence of sorbic acid (Table 2).

Development of toxin by C. parabotulinum types A and B in the presence of sorbic acid. The liver infusion and peptone broths containing 0.12 and 1.0% sorbic acid, respectively, were inoculated with 0.2 ml. of washed, detoxified (heated at 100°C., [212°F.] for 10 minutes) spore suspensions of *C. parabotulinum* type A and type B. All cultures as well as the uninoculated controls were incubated at 35°C. (95°F.) for 9 days.

Cell-free filtrates for injection were obtained by passing the cultures through Seitz centrifuge filters of pad porosity S-1. Aliquots of the filtrates were heated at 100°C. (212°F.) for 10 minutes to serve as detoxified controls. The bivalent botulinum (types A and B) antitoxin was prepared by the Jensen-Salsbery Laboratories, Kansas City, Missouri. The antitoxin was administered in 0.4-ml. amounts 30 minutes prior to injection of the unheated filtrate. Young male white mice (Webster strain) were used as the test animals. All injections were made by the intraperitoneal route. All filtrates were injected in 0.2-ml. amounts. The unprotected animals were observed continuously until death.

The results obtained indicated that the presence of 0.12 and 1.0% sorbic acid had no observed effect on the production of lethal toxin by the test cultures of *C. parabotulinum* type A or B. Typical neurotoxic symptoms were observed in the unprotected mice while the survivors exhibited no symptoms and were alive and healthy after 30 days. A higher level of toxin was produced in the basal peptone medium containing

sorbic acid than in the sorbic-free medium as evidenced by the time interval between injection and death; i.e., 180 minutes for sorbic-free controls as compared with 60 minutes for cultures grown in the presence of 0.12% sorbic acid. No adverse effects were observed in the test mice after intraperitoneal injection of 0.5 ml. of uninoculated media containing 0.12 and 1.0% sorbic acid.

Utilization of sorbic acid. In a previous paper *(18)* it was shown that a few isolates of *C. parabotulinum*, types A and B, utilized sorbic acid. The additional isolates of these 2 types tested during this investigation also utilized nearly all of the sorbic acid contained in the basal tryptone-yeast extract broth. Sorbic acid was determined by the spectrophotometric method of Melnick and Luckmann *(8)* after 7 days inoculation at 35°C. (95°F.). The apparent utilization (as measured by the disappearance of sorbic acid from the medium) by *C. parabotulinum*, type A, ranged from 86.4 to 100% with an average of 95.5% for 8 cultures. The apparent utilization of sorbic acid by type B cultures ranged from 86.6 to 100% with an average of 93.7% for 8 cultures. The one non-toxic strain of *C. parabotulinum* tested apparently utilized 62.2% of the sorbic acid present. *C. botulinum*, types C, D, and E (one culture of each type) did not utilize significant quantities of sorbic acid.

Uninoculated controls showed no loss of sorbic acid on incubation. Cultures grown in the absence of sorbic acid exhibited no absorption.

DISCUSSION

The first requirement should be that a food preservative considered to be relatively harmless as an animal or human dietary component provide adequate protection against the growth of food poisoning[c] bacteria. Because sorbic acid has been suggested for use with natural and processed cheeses having nearly neutral pH values and because it has received approval as a harmless dietary component *(15)*, the question of its effect on food poisoning organisms becomes most pertinent.

From the available data, reported here and elsewhere *(4, 7, 17, 18)*, it can only be concluded that sorbic acid is not a particularly effective inhibitor of food poisoning bacteria grown in a suitable medium. The *in vitro* resistance of *C. parabotulinum* and *C. botulinum* types to sorbic acid is obvious from the results recorded herein. Other food poisoning bacteria also exhibit resistance to sorbic acid. Cultures of *Streptococcus faecalis* (*a*-type hemolytic streptococci) grow in the presence of 0.12% sorbic acid in liver infusion *(4)*. Isolates of *Micrococcus pyogenes* var. *aureus (Staphylococcus aureus)* also are considerably more resistant to sorbic acid than other catalase positive microorganisms including species of *Salmonella (4)*. The staphylococci grow in 0.1% sorbic acid liver infusion medium when the pH value is in the proximity of 6.0 or above *(4)*. In contrast the species of *Salmonella* have been inhibited under all conditions studied until now. It should be clear that sorbic acid is more efficient under acid conditions (pH 4.5 and below). This is particularly true if the maximum concentration allowable shall be only 0.10% *(15)*. Otherwise, a very careful evaluation is required to determine the effects of such factors as moisture, concentration of soluble solids (salt, sugar, and organic acids), temperature, biological antagonisms, *et cetera*, on its efficiency in protecting each specific food product against food poisoning bacteria.

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[c] Used in the broad sense to include both food intoxications and food-borne infections caused by microorganisms.

SUMMARY

Recent interest in sorbic acid as a fungistatic agent prompted a study of its effect on the botulism organisms *C. parabotulinum*, types A and B, and *C. botulinum*, types C, D, and E. All of the isolates (18 type A, 16 type B, and 1 each of C, D, and E) grew in the presence of 0.12% sorbic acid in liver infusion without added phosphate (pH 5.6). The different cultures of *C. parabotulinum* all grew well and sporulated in the presence of 3.0% sorbic acid in liver infusion at pH 6.7 when vegetative cell inocula were used. Spores of all of the *C. parabotulinum* isolates germinated in the presence of 2.0% sorbic acid in liver infusion at pH 6.7. Growth of the different cultures in the acid range was not appreciably affected by the presence of 0.12% sorbic acid in liver infusion.

Filtrates toxic for mice were obtained when cultures of *C. parabotulinum*, types A and B, were grown in media with either 0.12 or 1.0% sorbic acid. Conventional use of botulinum antitoxin and heated filtrate controls indicated that botulinum toxin was produced.

Sorbic acid in concentrations of 0.12 or 1.0% in a suitable basal medium was utilized by all *C. parabotulinum* cultures tested.

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SORBITOL METABOLISM IN ALLOXAN-DIABETIC ANIMALS AS COMPARED WITH FRUCTOSE AND GLUCOSE[a, b]

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(Manuscript received July 16, 1954)

The metabolism of sorbitol has been investigated over a period of many years. The sustained interest in this sugar alcohol may be due to the fact that it has been abundantly used in foods and in medicine, and as a close relative of the hexoses, it can throw light on the metabolism of the sugars. Since the ingestion of sorbitol has apparently little effect on the blood sugar *(3)*, it has been recommended by various investigators from time to time as a substitute for sugar in the diabetic diet. However, no information is available regarding the exact insulin requirements of sorbitol. Consequently, we have investigated the metabolism of sorbitol in alloxan-diabetic rats in an attempt to evaluate the role of insulin in its oxidation. It was also desirable to include fructose in these studies since it has been suggested that fructose is a primary oxidation product of sorbitol *(1, 4, 9, 10)*. Glucose was also included for comparison purposes. In all of these studies oral feeding was resorted to, since it has been shown that neither sorbitol nor fructose can be oxidized by the muscle tissues in the absence of the liver and kidneys *(14, 15)*. In oral feeding practically all of the fed material must pass first through the liver before it is put into the general circulation, in contrast to parenteral injection in which a large fraction of the material is subjected largely to the excretory action of the kidneys before it is made available to the liver.

METHODS

The glucose and fructose used in these studies were uniformly labeled with C^{14} and made by biosynthesis *(13)*. The sorbitol was prepared by the reduction of the labeled glucose *(11)*. All radioactive determinations were carried out on a $BaCO_3$ obtained directly or by combustion or by direct count of the material, using a gas flow counter *(12)*.

Treatment of animals. Male albino rats weighing approximately 200 g. were made diabetic by the injection of alloxan monohydrate. Only those rats that excreted 15 to 25 g. of glucose per day over a 4-week period when fed a high carbohydrate diet were used. Two diets were employed in these studies, which are referred to as sucrose and fructose diets. Their compositions are as follows: (a) sucrose 68%, casein 15%, yeast 5%, salt mixture 4%, cod liver oil 5%, and Crisco 6%; and (b) sucrose was replaced by fructose with all other constituents the same. These diets were offered *ad libitum* during and at least 7 days before the feeding of the radioactive test substances which were administered orally at the dosage level of 1 g./kilo. Although 26 experiments were carried out, only 7 diabetic rats were used. Since the same rats were used from time to time, the fructose and sorbitol experiments were interspersed between the glucose runs. A minimum of 4 days was maintained between experiments so that there

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was no cross-contamination between the radioactive substances. In order to facilitate the collection of the expired air and urine, metabolism cages were employed.

RESULTS AND DISCUSSION

The rate of oxidation of sorbitol, fructose, and glucose as judged by the appearance of the administered C^{14} in the expired air is shown graphically in Figures 1 and 2 for the sucrose and fructose diets respectively. For comparison purposes, Figure 3 presents comparable oxidation data, taken from a previous publication *(11)*, for sorbitol and glucose in normal animals.

In Table 1 are the total recovery of the C^{14} in the CO_2 and urine for the diabetic animal experiments.

Although the data in Table 1 present, with respect to urine, only the total recovery of radioactivity, it should be pointed out that at least 90% of the C^{14} was found to be incorporated in glucose. This was proved in particular for the sorbitol fed

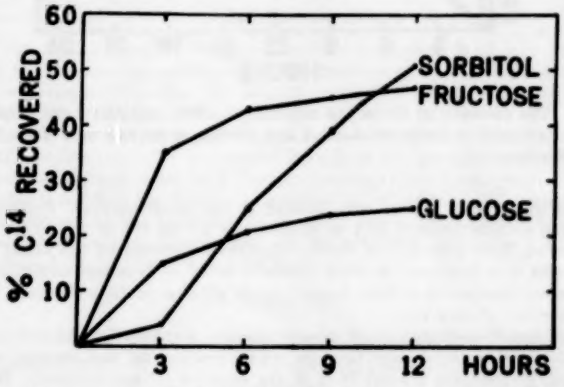

Figure 1. The recovery of C^{14} in the expired air after oral administration of C^{14} labeled sorbitol, fructose, and glucose to alloxan-diabetic rats maintained on a sucrose diet.

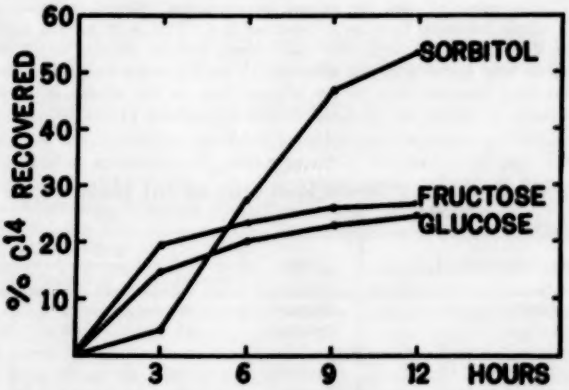

Figure 2. The recovery of C^{14} in the expired air after oral administration of C^{14} labeled sorbitol, fructose, and glucose to alloxan-diabetic rats maintained on a fructose diet.

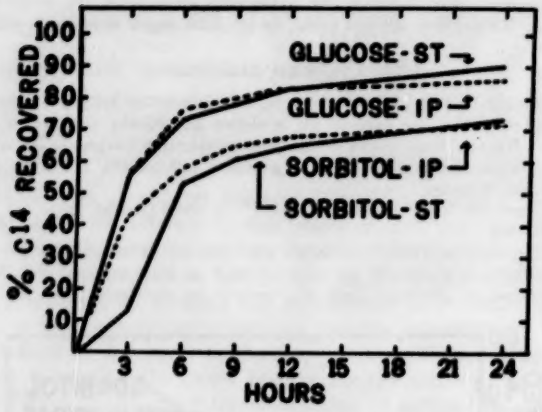

Figure 3. The recovery of C^{14} in the expired air after oral (ST) and intraperitoneal (IP) administration of radioactive sorbitol and glucose to normal rats. Data taken from previous publication *(11)*.

animals where the urinary glucose was isolated as the phenylosazone. Repeated crystallizations of the osazone showed that practically all of the C^{14} of the urine was in the glucose molecules. Less than 5% of the carbohydrates excreted by the animals receiving the fructose diet was fructose. A more accurate index of fructose excretion could not be obtained since glucose at a high concentration gives a positive fructose test by the colorimetric method of Roe *(7)*.

When the diabetic animals were placed on the sucrose diet, approximately 50% of the administered sorbitol and fructose was recovered in the expired CO_2 over a 12-hour period, whereas for glucose 26% of the ingested C^{14} was oxidized. With respect to sorbitol, it is seen that its total oxidation in diabetic animals approaches the values obtained in normal animals (Figure 3) over the same time period. After the animals have been on a fructose diet for a minimum of 7 days, their ability to oxidize fructose is no better than for glucose. The oxidation of sorbitol is not similarly affected by the fructose diet. It is apparent that the animal becomes less efficient in handling ingested fructose after being for some time on a fructose diet. This is in accord with numerous earlier studies with human subjects *(5)* and recent dietary studies using rats *(8)*. It is not clear as to why sorbitol is not affected by the fructose diet, since some workers have suggested that fructose may be an intermediate in the oxidation of sorbitol. It seems that sorbitol is either not oxidized to any significant extent by way of fructose

TABLE 1

Data showing the recovery of C^{14} twelve hours after the oral administration of sorbitol, fructose, and glucose to alloxan-diabetic rats

Substance administered	Diet	% C^{14} recovery		
		Expired CO_2	Urine	Total
Glucose (Av. 8 exps.)	Sucrose	26	71	97
Glucose (Av. 4 exps.)	Fructose	25	66	91
Sorbitol (Av. 2 exps.)	Sucrose	51	28	79
Sorbitol (Av. 4 exps.)	Fructose	54	31	85
Fructose (Av. 2 exps.)	Sucrose	47	51	98
Fructose (Av. 6 exps.)	Fructose	27	71	98

or, perhaps, the slow rate of intestinal absorption, which is characteristic of sorbitol, results in a slow and gradual formation of intermediates, which are oxidized more efficiently. However, in view of Blakley's *(2)* recent isolation from the rat liver of the enzyme dehydrogenase which converts sorbitol to fructose, it would appear very likely that sorbitol can be converted to fructose by the intact animal.

Evaluation of the alternate pathways of oxidation. A striking feature of these results is that the oxidation of sorbitol and fructose is so much greater than that of glucose when animals are on the sucrose diet. This indicates that at least two distinct pathways exist for the oxidation of sorbitol and fructose; these are (a) the oxidation after conversion to glucose and (b) the direct or alternative route (or routes) which does not involve conversion to glucose. The relative participation of these two pathways in these diabetic animals may be calculated for both fructose and sorbitol. The following is the calculation for the animals on the sucrose diet and administered fructose. If X = amount of fructose oxidized as glucose, then the amount of fructose changed to glucose = $X + 51$, since 51% of the fed fructose carbon was recovered in the urine as glucose carbons. Since 26% of the fed glucose carbons are oxidized, $\frac{X}{X + .51} = .26$ or $X = 18\%$. The total amount of fructose converted to glucose is $51 + 18$ or 69%. Thus, 31% of the fed fructose appears to have been oxidized after oral feeding by a pathway other than conversion to glucose. A similar calculation for sorbitol gives a value of 40% by routes other than through glucose.

It is obvious that insulin is required to handle efficiently the glucose formed from either sorbitol or fructose. The position of insulin in the alternative pathway (or pathways) is not certain and must wait further investigation. These results suggest that in the diabetic individual the feeding of sorbitol or fructose would increase the insulin requirement, but not to the same degree as would equivalent amounts of glucose. For an excellent review of the recent work on the alternate pathways for fructose, the reader is referred to the recent review by Racker *(6)*.

SUMMARY

The oxidation of sorbitol, fructose, and glucose has been examined in alloxan-diabetic rats. When the animals are placed on a 68% sucrose diet, approximately 50% of the administered sorbitol and fructose carbon is recovered in the expired air, whereas for glucose 26% is recovered. When the animals are maintained on a 68% fructose diet, fructose is no better oxidized than glucose, but the oxidation of sorbitol is not reduced. These results have been used to support the view that at least two pathways exist for the oxidation of sorbitol or fructose. An evaluation of the relative participation of sorbitol and fructose by these pathways is discussed.

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FACTORS ASSOCIATED WITH POTATO TEXTURE. I. SPECIFIC GRAVITY AND STARCH CONTENT[a]

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Among the various problems of potato technology, the texture of the cooked product has been accorded a considerable proportion of research effort. It is well-recognized that some varieties, after cooking, are glistening in appearance and feel more or less dry and granular on the tongue. These are characterized as *mealy*. On the other hand, other varieties have a translucent appearance when cooked and feel wet and pasty on the tongue. These are characterized as *soggy* or *waxy*.

There is often a considerable variation in texture within a given variety. In fact, striking individual differences have been observed among potatoes taken from the same hill *(9, 26)*; and different parts of a single potato will even vary in mealiness or sogginess *(30)*. Texture or so-called "cooking quality" has been shown in numerous investigations to be influenced by fertilizers *(3, 23)*, by maturity, by growing conditions generally (such as seasonal climate, region of growth, and physical condition of the soil), and by handling and storage conditions, etc. *(8, 9, 10, 29)*. Interest in the effect of these various growing and handling conditions, however, points up the fact that an understanding of textural differences must be based upon statistically established correlations between the chemical composition of a given sample and organoleptic and other aspects of texture. Thus, it has been found that a relatively high correlation exists between the degree of mealiness and the starch content of the raw tuber and between the degree of mealiness and specific gravity[b] *(5, 11, 12, 20, 25, 26, 31)*. Bewell *(2)* has even stated categorically that potatoes with less than 20% dry material will be of poor quality (*i.e.*, soggy) and those with more than 26% dry material will always be mealy.

Gilmore *(8)* and East *(7)* in attempting to explain the role of starch interpret mealiness as attendant upon the swelling and bursting of cells by the gelatinized starch within them. According to Whittenberger *(29)* and Whittenberger and Nutting *(30)*, textural variations are due to differences in internal pressure developed within cells by the swelling starch during cooking and to differences in the capacity of the individual cells to resist that pressure.

The regression coefficient of the starch-texture relationship has been found to be of the order of 0.3-0.6. This suggests that *the relative amount of starch* is not the sole factor in explaining texture and that an amplification of the concept is needed. Whittenberger *(29)* maintains that the relative amount of starch in individual cells has a greater influence on texture than

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[a] Presented at the Fourteenth Annual Meeting of IFT, Los Angeles, California, July 1, 1954.

[b] Specific gravity is a good index of starch content and has been useful as a non-destructive measurement of potato "quality."

does its concentration in the whole tissue. Briant *et al. (4)* found a correlation to exist between the size of the starch grains and texture — finding, specifically, that increased sogginess is associated wtih starch grain diameters below 0.02 mm. They also noted some general relationships between texture and the physical characteristics of the starch. However, no chemical analyses were made in their studies.

The present series of investigations has been undertaken to characterize more fully various aspects of potato texture. This first paper considers the relationship of specific gravity and starch content to texture. Emphasis is placed on the characteristics of potato starch in a wide range of varieties and on the effects of a standard processing method on starch in relatively homogeneous samples. Succeeding papers will deal with pectic materials, various physical measurements, and microscopic studies.

MATERIALS AND METHODS

Materials. For initial material selection, 15 potato varieties were obtained from different growing areas in the United States. In preliminary taste tests, 10 varieties were finally selected, representing 5 definitely *mealy* and 5 definitely *soggy* varieties. The varieties used were: *Cobbler* and *Triumph* (North Dakota Agricultural Experiment Station, Fargo, N. D.); *Bliss Triumph* and *Russet Burbank* (Idaho Agricultural Experiment Station, Aberdeen, Idaho); *White Rose* (University of California Agricultural Extension Service in Bakersfield, California); *Chippewa* and *Kennebec* (Maine Agricultural Experiment Station, Aroostook Farm, Presque Isle, Maine); *Russet Burbank* and *Green Mountain* (Utah Agricultural Experiment Station, Logan, Utah); and *Red Pontiac* (Wisconsin Agricultural Experiment Station, Spooner, Wisconsin).

Each variety was represented by a sample of 50 to 100 pounds. All samples arrived within a one-month period and were stored immediately at 45°F. Taste tests and physical and chemical experiments were carried out in a 1½-month period following receipt of samples.

Sampling and preparation. From the central region of each tuber, 4 longitudinal rectangular prisms (each 1 cm. × 1 cm. in cross section) were cut. This method served two purposes: it tended to mitigate the topographical variation in starch content and insured a uniform cooking time and uniform rate of heat penetration in each variety.

For taste tests, as well as for physical and chemical tests of cooked potatoes, the prismatic strips of potato were boiled for 10 minutes in distilled water. The ratio of water to potato was 2:1. Boiling was chosen over baking, because in preliminary tests panel members could distinguish textural differences more readily in the boiled samples.

Organoleptic tests. Eight persons were chosen as members of the taste panel after a training period of 2 weeks. In serving, a balanced, incomplete block design was used with randomized sample sets [Cochran and Cox *(6)*, Plan 11.15]. At each tasting session, 3 samples were served. The members of the panel were asked to rank the 3 samples for degree of mealiness in an ordinal arrangement of 1 to 3 (mealiest sample = 3, soggiest sample = 1). The criteria used for mealiness and sogginess were as described by Sweetman *(26)*. Each variety was tasted by the panel 9 times, giving a total of 72 judgments per variety, and each variety was served with every other variety in 2 combinations. The samples were served warm, without the addition of table salt or other seasoning.

Specific gravity. One hundred tubers of each variety were weighed in air and in water. From these measurements, the specific gravity was calculated by the usual procedure. It is necessary to point out that specific gravity values were determined for whole tubers, while only the central regions were presented to taste panels and analyzed in the other tests. The main purpose of the specific gravity measurements was to check the practicality of this method of predicting potato texture. However, the rest of the tests were devised to yield information on the mechanism of textural behavior.

Dry weight. Strips from the central portions of 25 tubers of each variety, both raw and cooked, respectively, were weighed, transferred with a known amount of distilled water into a Waring blender, and blended for 5 minutes. One hundred g. of the slurry,

which contained about 10 g. of potatoes, were transferred into weighed aluminum dishes and dried first on a steam bath and then in a vacuum oven at 70°C. for 48 hours. (The rest of the slurry was used for the analysis of starch and sugars.) After drying, the aluminum dishes were put into a desiccator for 24 hours and subsequently weighed. The percentage of dry weight was calculated on the basis of the original raw, or cooked, potato slices.

Total starch. Part of the slurry, which was used in the determination of the dry weight, was analyzed for starch. This slurry aliquot was extracted 4 times with 80% ethanol. Determination of total starch was carried out by the anthrone method described by McCready *et al. (15)*. The starch content was calculated on a fresh weight basis.

Amylose and amylopectin. Amylose and amylopectin content was estimated colorimetrically with iodine according to the method of McCready *et al. (15)*. Pure amylose and amylopectin were prepared from Russet Burbank (Idaho) potatoes according to the method given by Schoch *(22)*. The standard curve obtained by mixing these 2 components in different ratios, with a total concentration of 0.001%, was somewhat different from that obtained by McCready and Hassid *(16)*. Although the values for pure amylose were about the same in both curves, in the curve of McCready and Hassid pure amylopectin had a color intensity of 50 while the figure for pure amylopectin here was 25 Klett units. These differences may be due to different methods of preparation. The lower value here indicates that this amylopectin preparation was somwhat purer than that of McCready and Hassid.

For the estimation of amylose and amylopectin in potatoes, the same sugar-free starch preparation was used as in the determination of total starch. This was diluted to a concentration of 0.001%, and the procedures of McCready *et al. (15)* were followed.

Sugars. Sugars were determined in the combined ethanolic extracts used to remove the sugars from the samples of the total starch determination. The ethanol was evaporated from these extracts, and HCl was added to bring the solution to a 0.1 N strength. This solution was boiled for 10 minutes to hydrolyze the sugars to monosaccharides. A volumetric modification of Fehling's method was used *(13)*. Although the hydrolyzed sugars were not identified chromatographically, the results were reported as glucose on the basis of a standard curve.

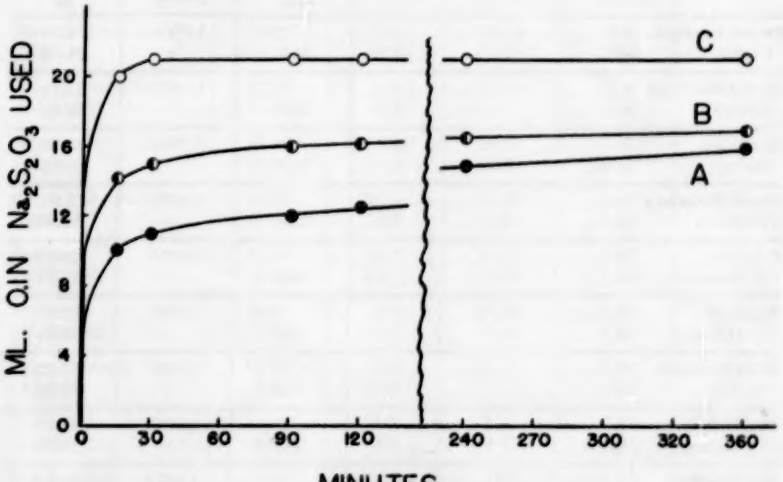

Figure 1. Activities of (A) alpha-amylase, (B) beta amylase, and (C) equal weights of alpha-amylase and beta-amylase. The total enzyme concentration for each was 0.05 g. enzyme preparation per 100 ml. solution. The substrate consisted of 1.3% gelatinized starch of Russet Burbank (Idaho) potato at pH 5.7. The reactions were carried out at 25°C.

Starch hydrolysates by preparations of amylase. Pure alpha-amylase and beta-amylase* were obtained, and the course of the reactions catalyzed by these enzyme preparations were measured (Figure 1). After 360 minutes, in experiments with alpha-amylase and with alpha- and beta-amylase, no alcohol-precipitable starch was left. In the experiment with beta-amylase, 0.3% non-hydrolyzed dextrins remained from a substrate of 1.3% gelatinized potato starch.

Hydrolysates by beta-amylase and by alpha + beta-amylase were analyzed in aliquots of the same slurry used for the dry weight and starch analyses. The aliquots were subjected to autoclaving at 120°C. for one hour to achieve complete gelatinization. Before autoclaving, the samples were diluted to an approximate concentration of 1% starch. The autoclaved samples were cooled, and enzyme solutions were added to achieve a final concentration of 0.05% of the enzyme preparations. Time of enzymic hydrolysis was 12 hours. At the end of the hydrolysis, aliquot samples were taken for sugar analysis in the manner described in the preceding section, except that no acid hydrolysis was applied to convert the sugars to monosaccharides. A standard curve for maltose · H_2O was determined. The percentage of starch hydrolyzed enzymatically was reported as 0.9 × maltose · H_2O percentage minus the starch equivalent of free sugars (0.9 × free sugars reported as glucose). This subtraction was necessary because the substrate of the enzymatic hydrolysis also contained free sugars.

EXPERIMENTAL RESULTS

Organoleptic scores. The total ranking value of each variety has been taken as its score. Since each variety was tasted 9 times by 8 members of the panel, the highest

* From the Nutritional Biochemical Corporation, Cleveland, Ohio.

TABLE 1

Organoleptic score, specific gravity, and dry weight in raw and cooked potatoes

Variety		Organoleptic score	Specific gravity	Dry weight %
Russet Burbank (Utah)	r[1]	194	1.0968	24.36
	e[2]			21.36
Russet Burbank (Idaho)	r	183	1.0871	21.44
	e			20.62
Kennebec (Maine)	r	169	1.0964	23.23
	e			20.63
Green Mountain (Utah)	r	168	1.0958	20.91
	e			16.99
Cobbler (N. Dakota)	r	162	1.0835	19.36
	e			17.50
Triumph (N. Dakota)	r	130	1.0784	17.52
	e			15.91
Bliss Triumph (Idaho)	r	115	1.0747	24.88
	e			21.05
White Rose (California)	r	112	1.0680	18.70
	e			16.79
Red Pontiac (Wisconsin)	r	107	1.0577	13.91
	e			13.08
Chippewa (Maine)	r	102	1.0694	16.22
	e			13.97

[1] r = raw.
[2] e = cooked.

possible total score would be 216 for the mealiest variety, and the lowest possible total score 72 for the soggiest variety. The median point is 144, and the total range, 92. From Tukey's *(27)* table, at 5% risk, the least significant difference is 21.28 total score points, and at 1% risk, it is 28.88 total score points.

The organoleptic scores are given in Table 1, and the varieties are listed in the order of degree of mealiness. On the basis of the above statistical analysis, the difference between the mealy (Russet Burbank from Utah and Idaho, Kennebec, Green Mountain, and Cobbler) and the soggy (Triumph, Bliss Triumph, White Rose, Red Pontiac, and Chippewa) groups is highly significant. There even appear to be statistically significant differences between some of the varieties within each group.

Specific gravity. Specific gravity values are shown in Table 1. The gross regression coefficient between texture score and specific gravity is 0.826, which is highly significant (1% level) according to the "t" test. The square of the regression coefficient, or the coefficient of determination, is 0.6824, indicating that about 68% of the variation in the taste panel scores can be explained by the variation in specific gravity.

This correlation is especially notable when it is recalled that the specific gravity measurements were performed on whole potatoes whereas only the interior sections of the potatoes, which definitely have lower specific gravity values than the whole tubers *(30)* were tasted.

Dry weight. The dry weight values (Table 1) generally follow the trend of the specific gravity. However, in the case of Bliss Triumph, a larger part of the dry weight may have been contributed by compounds of lower density than starch, possibly cellulose and hemicellulose.

TABLE 2

Results of starch analysis in raw and cooked potatoes

Variety		% Total starch	% Amylose in starch	% Starch hydrolyzed by $\alpha + \beta$ amylase	% Starch hydrolyzed by β amylase	% Sugar
Russet Burbank (Utah)	r[1]	19.06	16.5	18.96	10.22	1.07
	c[2]	15.92	11.5	15.67	10.62	0.66
Russet Burbank (Idaho)	r	17.05	14.0	16.06	11.12	0.46
	c	15.85	10.8	15.78	12.00	0.36
Kennebec (Maine)	r	17.95	7.5	16.87	9.67	1.23
	c	15.21	10.4	14.85	10.38	0.79
Green Mountain (Utah)	r	16.21	27.5	16.58	7.38	1.49
	c	11.40	10.6	11.56	5.85	0.89
Cobbler (N. Dakota)	r	13.48	15.5	13.31	9.57	0.09
	c	9.42	14.0	8.76	5.34	0.54
Triumph (N. Dakota)	r	10.90	9.7	10.76	6.43	1.52
	c	8.55	13.5	8.03	4.22	0.74
Bliss Triumph (Idaho)	r	12.75	9.0	12.56	7.94	3.20
	c	8.42	10.1	7.51	4.08	2.10
White Rose (California)	r	12.20	21.5	12.02	6.63	1.20
	c	9.70	9.7	9.42	4.42	0.85
Red Pontiac (Wisconsin)	r	7.12	5.0	7.03	4.18	2.33
	c	6.25	12.0	6.39	4.93	1.06
Chippewa (Maine)	r	12.82	9.5	12.32	8.65	1.44
	c	7.75	11.0	6.93	5.23	1.36

[1] r = raw.
[2] c = cooked.

Total starch. The total starch content of the raw potatoes also tended to follow the trend shown by the specific gravity (Table 2). However, in three cases, Green Mountain, Bliss Triumph, and White Rose, there is a certain deviation from this trend. The regression coefficient of the organoleptic score with the starch content of the raw potatoes is higher (0.848) than that with the specific gravity.

On the other hand, there is even a higher correlation between the total starch content of the cooked potatoes (Table 2) and the organoleptic score. The gross regression coefficient is 0.883, which is highly significant. This value indicates that the starch content alone, in the cooked potatoes, may explain 78.04% of the variation of the taste panel score. This result would suggest that the amount of starch in the potato tissue at the time of its consumption is largely responsible for the texture observed by the individual taster.

The decrease in the total starch content during the cooking process (Table 3) does not show any clear-cut relationship with texture scores. This decrease is due mostly to the diffusion of the starch solution from the cells to the cooking medium. (In Cobbler potatoes, 97.3% of the lost starch was located as such in the cooking medium.) Diffusion is made possible by the change in permeability of the cell membranes and by the increased solubility of the starch on heating. Personius and Sharp *(18)* have shown that a change in permeability takes place at the temperature of starch gelatinization. They have also observed a difference in permeability between soggy and mealy potatoes. If cell permeability alone governs the diffusion of starch from the cell, there should thus

TABLE 3

Some properties of potato starches calculated from Table 2

Variety		Amylose g./100 g. potato	Amylopectin g./100 g. potato	Outer branches of amylopectin g./100 g. potato[3]	Outer branch % of amylopectin	Decrease in starch by cooking g./100 g. potato[4]
Russet Burbank	r[1]	3.14	15.92	7.08	44.47	
(Utah)	e[2]	1.83	14.09	8.79	62.38	3.14
Russet Burbank	r	2.39	14.66	8.73	59.55	
(Idaho)	e	1.72	14.13	10.28	72.75	1.20
Kennebec	r	1.35	16.60	8.32	50.12	
(Maine)	e	1.60	13.61	8.78	64.51	2.74
Green Mountain	r	4.46	11.75	2.92	24.85	
(Utah)	e	1.20	10.20	4.65	45.59	4.81
Cobbler	r	2.09	11.39	7.48	65.67	
(N. Dakota)	e	1.32	8.10	4.02	49.62	4.06
Triumph	r	1.04	9.86	5.39	54.66	
(N. Dakota)	e	1.15	7.40	3.07	41.48	2.35
Bliss Triumph	r	1.15	11.60	6.79	58.53	
(Idaho)	e	0.84	7.58	3.24	42.74	4.33
White Rose	r	2.62	9.58	4.01	41.85	
(California)	e	0.94	8.76	3.48	39.72	2.50
Red Pontiac	r	0.36	6.76	3.82	56.50	
(Wisconsin)	e	0.65	5.50	4.28	77.81	0.87
Chippewa	r	1.22	11.60	7.43	64.05	
(Maine)	e	0.85	6.90	4.38	63.47	5.04

[1] r = raw.
[2] e = cooked.
[3] Outer branches of amylopectin g./100 g. potato were calculated as: starch hydrolyzed by beta-amylase minus amylose, both in g./100 g. potatoes.
[4] Decrease in starch content by cooking is the difference between the starch content of the raw and the starch content of the cooked potatoes.

be a direct relationship between the texture characteristics of the potatoes and the relative decrease in starch content. Since this is not the case here, the amount of starch diffusion must be determined by additional factors.

Amylose and amylopectin content. Neither the amylose nor the amylopectin content of the starch is correlated with taste scores. In Table 2, only the percentage of amylose in the starch is given. The amylopectin percentage may be obtained by difference (100 − % amylose). The amylose percentage varies from 27.5%, in the case of Green Mountain, to 5% in Red Pontiac. The amylose percentage in the starch decreases during the cooking in cases where the amylose percentage of the raw potato starch is above 10%; and where it is below this value, it increases.

Starch hydrolyzed by alpha + beta-amylase. The amount of enzyme-hydrolyzed starch approximates the total starch content (Table 2). In each case, except for the Green Mountain, a small amount of "limit dextrin," which varies from 0.09 to 1.08 g./100 g. potato in the raw tubers, is unhydrolyzed. In the Green Mountain potatoes, the amount of enzyme-hydrolyzed starch is greater than the amount found by the anthrone method. The same is true in the cooked potatoes of this variety.

Starch hydrolyzed by beta-amylase. Beta-amylase can hydrolyze amylose molecules and the outer branches of amylopectin until it reaches a 1-6 glycosidic linkage. Therefore, from the amount of starch hydrolyzed by beta-amylase (Table 2) and from the amylose content (Table 2), it is possible to estimate the amount of outer branches of the amylopectin. The data are presented in Table 3. The values found for the outer branches of amylopectin are in the neighborhood of those found by Meyer and Gibbson *(17)* for corn amylopectin.

None of the characteristics of the structure of the starch examined in this investigation seems to have any correlation with potato texture.

Sugar: The sugar content of the tubers, calculated as glucose, varies from 0.09 to 3.20% in raw samples (Table 2). During cooking, except in the case of the Cobbler variety, the sugar content decreases. No relation seems to exist between sugar content and potato texture.

DISCUSSION

The purpose of the first phase of this investigation has been to relate texture with specific gravity and with the properties of starch in various potato samples at the time of the organoleptic tests. However, it should be stressed that the results of the analysis are not necessarily specific for each variety investigated. No attempt has been made to invoke the history of the potatoes, *i.e.*, the growing and storage conditions which influence the composition of the raw material. Therefore, this investigation relates texture with its most immediate causative aspect, the composition of the potato tuber.

Limiting the scope of effects to the last and immediate ones logically leads to the analysis of the properties of the cooked potatoes. This approach has been neglected for the most part since major interest has centered on the prediction of the texture of processed potatoes from the raw tubers. Although the main purpose of this investigation has been to find textural correlations, the very nature of the approach has brought forth fundamental information on the effects of a standard processing upon chemical composition.

The organoleptic scores are taken as the criteria for mealiness or sogginess. Because of the individuality of each taster, high fluctuations might have been expected among the individual judgments. Surprisingly, each presentation of samples resulted in agreement of a large majority, and in some cases, unanimous decision was reached. The statistically significant values for the sample groups should be considered in light of the fact that

within a sample there are large variations in texture and chemical composition, which tend to decrease the differences among the varieties.

Although the specific gravity-texture correlation is significant, it is probably meaningful only insofar as it reflects the starch-texture correlation. Specific gravity can be, and has been, useful in predicting the expected processed texture of a batch of raw potatoes *(11)* and may even be used to separate tubers into different quality classes *(14, 24)*. Like starch content, it depends on variety and on cultural and storage conditions *(29)*.

The role of starch content as the main determining factor in potato texture is confirmed in this investigation. Even though the correlation of the starch content of raw potatoes with texture ($r = 0.848$) is highly significant, the correlation is improved if calculated on the basis of the starch content of the cooked potatoes ($r = 0.883$). The high value here may be due to the pronounced varietal differences. This result also indicates that the amount of starch found in cooked potatoes is dependent not only on the starch content of the raw potatoes but also on other factors, such as the solubility of the heated starch and the permeability of the cell wall and cell membranes during processing. Permeability is important because loss of starch occurs by diffusion into the cooking medium. It has been found by Potter and Hassid *(19)* that no detectable depolymerization occurs in starch molecules during a mild treatment like boiling for a short time. This has also been confirmed by Baum and Gilbert *(1)*, who made viscosimetric determinations on amylose and amylopectin of stored potatoes after 5 minutes of boiling. They could detect no change in the intrinsic viscosities.

The percentage of amylose in potato starch decreases during cooking in cases where the amylose percentage of raw potato starch is above 10%, and where it is below this value, it increases. It might be supposed that amylose, being a smaller molecule than amylopectin, would always diffuse more readily from the cell. The proportional decrease of amylose during cooking perhaps may be explained on the basis of simple diffusion. However, where the amylose percentage appears to increase proportionally, there may have been sufficient loss of amylopectin by diffusion and/or by initial enzyme activity to produce a spuriously higher amylose-amylopectin ratio. Although the amylose content of potato starch is generally given as 20%, the values here are in some cases considerably lower. It is possible that these values are due to analytical or sampling error. However, it seems more likely that the general tendency towards low amylose content is a characteristic of the starch in the central region of the tuber.

Reeve *(21)* has observed that White Rose potatoes lose more amylose in cooking than Russet Burbank potatoes. This observation is based on the development of blue color in the cooking medium upon the addition of iodine. Although the data here confirm Reeve's finding, this varietal difference is not characteristic of the difference between mealy and soggy potatoes. In the present investigation, it has been found that some mealy potatoes, such as the Green Mountain variety, lose relatively more amylose than some soggy potatoes, such as the Red Pontiac variety.

The relative amount of amylose or amylopectin in the different potato samples is not correlated with the texture scores. It is quite possible that a structural difference exists between mealy and soggy potato starches,

but this difference is not observable by the iodine sorption technique. Ulmann *(28)* has shown that both the amylose and the amylopectin fractions can be further fractionated in an aluminum oxide column, and that these fractions give different shades of color with iodine. Ulmann's fractions have not yet been characterized chemically. But in view of present-day knowledge of starch composition, it can be stated that there are no differences in the composition of starch which can be related to texture in cooked potatoes.

SUMMARY

Ten potato varieties were evaluated for their boiled texture organoleptically. Specific gravity and the starch content of raw tubers were significantly correlated with texture scores. The correlation between the starch content of cooked potatoes and the organoleptic scores was highly significant.

The chemical nature of starch in these potato samples was characterized. During the cooking process, total starch invariably decreased, but the amylose content decreased in some varieties and increased in others. Possible explanations of this behavioral difference, on the basis of diffusion and enzyme activity were considered. No relationship exists between the chemical nature of the starch and potato texture.

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NEWER CONCEPTS OF THE ROLE OF FATS AND OF THE ESSENTIAL FATTY ACIDS IN THE DIET*

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The concepts that fats are to be regarded as optional components of the diet is widely held among food technologists, dietitians, home economists, nutritionists, and other food specialists, as well as by the laity. However, within the last few years there has been increasing evidence that fats should be regarded as obligatory constituents in our diet. The realization that fats are essential nutrients has been developed as a result of the demonstration of the increasing number of functions to be ascribed to the so-called "essential fatty acids." However, it is not certain at the present time whether the presence of the essential fatty acids in fats is responsible for all of the specific nutritional effects of fats, or whether other properties of this foodstuff may account for some of them.

Fats have the known distinction of possessing the highest caloric density of any of the foodstuffs. Thus, one gram of fat contributes something over 9 large Calories while one gram of protein or carbohydrate yields only about 4 large Calories. Actually, the ratio of caloric values of fats: proteins or fats: carbohydrates in natural foodstuffs is usually greater than the 2.3:1 ratio indicated by the above values. This is because fats are usually stored without water since they are hydrophobic, while proteins and carbohydrates most often occur naturally with considerable quantities of water.

Certain fats serve as sources of the fat-soluble vitamins [vitamin A, D, and E (tocopherols)] while all fats can function as solvents for them and so assist in their absorption. The adipose tissue serves as an insulating agent under the skin, thus preventing unduly rapid loss or gain of heat. Such fatty tissues also act to protect large organs such as the kidneys from injury by forming a protective layer around them; moreover, fat likewise protects the smallest nerves and similar structures which are imbedded in it from injury. From a physiological standpoint, probably the most important function of fat is as a reserve foodstuff.

Fats are essential in creating an appetizing diet. Low-fat diets become extremely monotonous and unpleasant when continued over any considerable period. According to Starling *(72)*, the lack of fat in the diet was the chief cause for the voluntary underconsumption of food in Great Britain during World War I, with the attendant general loss in weight of the population. Finally, from a culinary standpoint, fats have great

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importance. The preparation of many of our baked foods would be impossible without shortening and, of course, no fried foods can be prepared without fats. Although all of these facts tend to emphasize the desirability of fats in the diet, they do not prove the indispensability of this foodstuff from a nutritional viewpoint.

NUTRITIONAL INDICES WHICH INDICATE THAT FATS IN GENERAL ARE ESSENTIAL

There are a number of nutritional indices which suggest that fats in general are indispensable. These include not only the classical procedures for evaluation such as growth, pregnancy, and lactation, but also some new ones such as the time of sexual maturity, the nitrogen-sparing action, work capacity, survival during fasting, and the ability to combat external stresses.

1. Growth. The ability of fat-containing diets to provide a greater gain in weight for growing rats than fat-free regimens has been repeatedly demonstrated. Thus, Hoagland and Snider *(43)* observed greater gains-in-weight in rats on diets containing 30% or 55% of lard than in one having 5% of this fat. In another series of tests with diets into which 5, 15, 30, or 54% of lard was incorporated, it was found that the animals receiving the 30% fat diet did best *(42)*. Deuel and associates *(23)* have reported that when male rats were fed on diets containing 0, 5, 10, 20, or 40% of cottonseed oil, those groups receiving 20 or 40% of cottonseed oil exhibited the greatest increases in body weight. When the fat under investigation was a vegetable margarine fat, the optimum diet appeared to be one which contained 30% of fat. It is suggested on the basis of these data *(19)* that, in the case of the rat, the optimum fat level in the diet is 30% by weight which corresponds with 50% of the total calories.

Since *ad libitum* feeding was employed in the above tests, it is uncertain as to whether the greater body weights found in the rats receiving the high-fat diets are to be ascribed to improved nutrition in these groups or simply to the fact that the diets containing the high-fat content permitted a greater caloric intake than was possible with low-fat or fat-free regimens. This question has been answered by the paired-feeding tests. Thus, Forbes *et al. (31)* observed that the gains-in-weight of young rats on isocaloric diets containing 2, 5, 10, or 30% of fat (mostly lard) became progressively better as the proportion of fat was increased; similar results were noted in mature animals *(32)*. It was subsequently recorded by Forbes and co-workers *(33, 34)* that the increasing efficiency of the high-fat diets was related to a decrease in total heat production. In confirmation of these results, French *et al. (35)* have noted that a better energy utilization obtains on a high-fat diet (30%) than on a low-fat regimen (2%), when the protein level was reduced from 22% to 7%. The most recent data from the same group *(36)* have indicated that an identical growth can be brought about in male and female rats on a high-fat diet with total caloric intakes 4.8% and 5.7%, respectively, less than was required with the low-fat regimen. Undoubtedly, the greater economy of fat under these diverse conditions is related to the lower specific dynamic action with protein or carbohydrate which has been called the "associative dynamic effect" *(30)*.

The growth response has likewise been shown to excel in the case of high-fat diets when the test animals have previously been subjected to undernutrition. Thus, in the case of weanling rats, which had been stunted by caloric restriction for 12 weeks, Scheer and collaborators *(67)* found that when the depleted rats were fed *ad libitum* on diets containing 0, 5, 10, 20, or 40% of fat, the groups receiving the three highest levels of fat did equally well and were far better than the other groups. However, the highest potential for growth still remaining after 12 weeks of *ad libitum* feeding was found in the group receiving the 40% fat diet; by this time, practically no gain-in-weight was being obtained by the rats on the 0% or 5% fat diets. In other tests by Scheer *et al. (65)* with young adult rats subjected to severe caloric restriction, it was observed that the decrease in body weight and the mortality rate were least for those animals on the high-fat regimen. One can only conclude on the basis of growth tests that fats possess a characteristic growth effect.

2. Time of maturity. The age at which sex maturity occurs provides another physi-

ological index of nutritional value. Although this phenomenon has been believed to bear some relationship to dietary conditions, it has recently been demonstrated to be directly related to the level of fat intake in rats *(23)*. Thus, while 81 days was required before the opening of the vagina in female rats on a fat-free diet, this occurred at the following ages in rats receiving fat diets: 5%, 70 days; 10%, 68.5 days; 20%, 65.8 days; and 40%, 65.1 days. In tests in which rats were fed diets for 12 weeks after weaning so restricted in calories that growth did not take place, sexual maturity did not obtain, irrespective of the level of fat in the diet *(67)*. However, when the rats were allowed to consume the several diets *ad libitum*, sex maturity was observed in the following average periods: 0% fat, 14.7 days; 5% fat, 11.9 days; 10% fat, 10.5 days; 20% fat, 7.7 days; and 40% fat, 9.8 days *(67)*. It is not certain whether the progressive effect of increasing doses of fat in producing sex maturity at earlier periods is a direct action of fat or an indirect one attributed to the increased rate of growth.

3. Pregnancy and lactation. Growth, pregnancy, and lactation are believed to provide increasingly critical indices of nutritional value. The beneficial effects of fat under these conditions is now believed to be traced largely, if not entirely, to their essential fatty acid content. This is discussed later.

4. Work capacity. Until recently, it has been generally considered that carbohydrate provides a better source of energy for muscular work than does protein or fat. The Hill-Meyerhof theory which embodies the glycogen $\rightleftarrows$ lactic acid interrelation in muscular contraction postulates carbohydrate as the source of such energy. Moreover, Krogh and Lindhard *(50)* reported that, although fat could serve as a source of energy for muscular contraction in man, it was 10% less efficient than carbohydrate. This finding is one which is opposed to an earlier study of Anderson and Lusk *(6)* with dogs; in the latter case, the energy expenditure was identical irrespective of the foodstuff which served as the source of the calories.

Not only may fat serve efficiently as a source of energy for muscle work, but some recent experiments in the laboratory of the author *(23)* have indicated that it may provide more effectively than does carbohydrate. Using a swimming test devised by Scheer *(66)*, as a method for providing exhausting exercise, it was found that the average duration over which rats could swim could be considerably extended if they had been receiving high-fat diets. The average periods of swimming of 3 series of rats receiving diets containing cottonseed oil were as follows: 0%, 654 seconds; 0% but containing 1% linoleate, 636 seconds; 5%, 863 seconds; 10%, 849 seconds; 14%, 1021 seconds; 20%, 1044 seconds; and 40%, 1044 seconds. Similar variations in performance were observed with rats which had received different levels of margarine fat in the diet. Although Ershoff *(25)* has recently failed to demonstrate a similar effect in rats and mice, the results are not directly comparable since the procedures employed were entirely different from those described by Scheer *et al. (66)*.

Confirmatory evidence of the increased physical capacity as related to fat diets has been obtained by an entirely new procedure by Samuels and co-workers *(64)*. When rats were fasted which had previously been maintained over a 28-day period on diets in which 80% of the calories were derived from protein, carbohydrate, or fat, the total forced activity during the period of survival was greatest with those rats previously on the 80% fat regimen. The average work records were as follows: fat group, 19,700 $\pm$ 1780 cage revolutions; carbohydrate group, 13,800 $\pm$ 1630 cage revolutions; and the protein group, 6465 $\pm$ 1650 cage revolutions. Although the previously fat-fed rats survived longer, it was not believed that the better performance by this dietary group could be ascribed solely to that fact.

5. Nitrogen retention. The ability of a foodstuff to spare protein has long been regarded as an important index of its nutritional value. Nitrogen equilibrium can readily be established when carbohydrate is present in the diet, but fat has little effect on lowering the protein requirement. In fact, Lusk *(52)* has reported that, on a constant protein intake, urinary nitrogen was depressed in man by the ingestion of carbohydrate but not by that of fat. Carbohydrate therefore would appear to function best in sparing *exogenous* protein metabolism.

However, it has recently been demonstrated that fat plays a commanding role in *endogenous* protein metabolism. When the nitrogen excretion of rats was reduced to the "wear and tear" quota by the administration of a protein-free diet, it could be maintained at this low level only when generous amounts of fat were supplied *(74)*. No

differences could be observed in the level of urinary nitrogen on protein-free diets if they were administered at 100% or 75% of the caloric requirements, irrespective of whether or not fat was present in the diet. However, when the caloric intake was reduced to 50% of the required level, nitrogen elimination was maintained at a low value only in the case of the animals receiving the protein-free diet containing 20% of fat. The variations in the protective effect of fat over non-fat diets were further exaggerated when the caloric intake was reduced to 25% of the basal requirement. Schwimmer and McGavack *(71)* have demonstrated a similar protective action of fat on the "wear and tear" quota of human subjects on a fat-free diet. This indicates that the role of fat in this condition is a more or less general one.

In addition to the behavior of fat in relation to the nitrogen minimum, Samuels and co-workers *(64)* discovered another condition in which this foodstuff spares protein. When rats were previously conditioned by being fed on an 80% fat diet for 28 days, the nitrogen excretion during the subsequent fast period was much lower than when the rats had received carbohydrate or protein at a level of 80% during the prefast conditioning period.

6. Period of survival. Longevity and the period of survival during fasting offer two related indices for comparing the nutritional value of foodstuffs. In the tests carried out by Samuels and collaborators *(64)*, the survival of rats during a fast was longest if they had been prefed on a fat diet. Thus, the average period of survival was observed to be 10.2 ± 1.68 days for the rats receiving the protein diet in the prefast period, while it was 15.5 ± 0.46 days for the group in which carbohydrate made up the prefast regimen. Compared with these results, the rats which had been prefed on fat survived for an average of 18.3 ± 0.44 days.

The data on the effect of the level of dietary fat on longevity are somewhat controversial. On the one hand, Carlson and Hoelzel *(13)* reported that rats receiving an omnivorous diet containing 28% of fat and 35% of protein lived longer than did rats which received a vegetarian diet containing 8% of fat and 30% of protein but supplying more protein and roughage on an isocaloric basis. However, because of variations in the diet, it is difficult to attribute the variations in longevity in the aforementioned experiments solely to variation in the fat content. On the other hand, French *et al. (36)* observed that the life span of male rats was shorter on a high-fat diet (22.7%) than on a low-fat regimen (3.4%). In the case of females, no decisive differences were noted.

7. In conditions of stress. Fats have been shown to function in assisting the animal to meet such internal stresses as are occasioned by growth, pregnancy, lactation, work, and the maintenance of protein stores. Another index for establishing the usefulness of fat in the diet is whether or not it aids in a specific manner when the animal is subjected to such external forms of stress as thyrotoxicosis, x-irradiation, cold, and hepatectomy.

(1) Thyrotoxicosis. Fat has repeatedly been found to be the food from which the greatest beneficial effect is derived in hyperthyroidism. Abelin *et al. (1-3)* were the first to note this effect; high-fat diets were found to counteract the increased metabolism and to prevent the rapid depletion of liver glycogen usually associated with the administration of the thyroid hormone.

Although some workers have attempted to correlate the beneficial effect of fat in thyrotoxicosis with its essential fatty acid content, several recent reports have tended to make this suggestion open to question. Thus, Ershoff *(24)* found that the growth retardation in rats given thyroid was counteracted equally well by a number of fats of widely different essential fatty acid content. Greenberg and Deuel *(39)* reported that the presence of cottonseed oil in the diet not only permitted normal growth when thyroid powder was given but likewise prevented mortality. In later tests, Greenberg *(37)* was able to point out a protective action against thyroid exhibited independently by linoleic acid and by cottonseed oil. It is now believed that the beneficial effect of fat may be partly attributable to the essential fatty acid content, the requirements for which might be increased in hyperthyroidism, and partly to the higher caloric density of high-fat diets, which enable hyperthyroid animals to meet their caloric needs. In any event, the protective effect of fat in hyperthyroidism is one of the most striking examples of the beneficial action of this foodstuff.

(2) X-Irradiation. Since the protective action of fat against x-rays seems to be related to its essential fatty acid content, this subject will be discussed later.

(3) Cold Stress. Cold offers a comparatively new form of stress for assisting in studies of nutrition, since it produces a marked increase in metabolic level. Mitchell and co-workers *(57)* have observed that general psychomotor performance and visual efficiency were maintained better in human subjects exposed to severe cold when a high-fat diet was ingested, than when a high-carbohydrate diet was employed. The former diet likewise allowed a maximum speed in tapping tests. It was also noted that tissue temperatures were maintained better in subjects on the high-fat diets than in those on the high-carbohydrate regimens; this difference is believed to be due to a lowered heat emission rather than to an increased heat production in the individuals receiving the high-fat diets.

(4) Hepatectomy. Since the liver is responsible for mediating so many metabolic functions, any interference with its function results in a critical form of stress. Although it is impossible to maintain life in dogs for much over 8 hours when the whole organ is removed, it is possible for animals to recover when as much as 70% of the organ has been extirpated. In animals subjected to such an operation, there is therefore an opportunity to study the effect of diet on the rate of regeneration of liver tissue.

Although Brues *et al. (9)* reported that the rate of regeneration of the liver of partially-hepatectomized rats was slower when fat was the chief dietary component, as compared with the rate when protein or carbohydrate was the principal dietary constituent, Rogers and co-workers *(62)* have observed that moderately high-fat diets served as efficaciously in permitting the regeneration of liver tissue as did low-fat regimens. In fact, when the intake of rats on a 30% fat diet was limited to that of rats on a 3% fat diet, a significantly higher increment in liver protein and in body weight obtained in the high-fat group. When an *ad libitum* feeding was employed, the improvement of the rats on the high-fat regimen over those on the low-fat diet was considerably greater than in the paired-feeding tests.

NUTRITIONAL INDICES WHICH INDICATE THAT ESSENTIAL FATTY ACIDS ARE REQUIRED

The first investigation, which proved that the rigid exclusion of fat from the diet results in a fat-deficiency syndrome, was that of Evans and Burr *(26)* in 1926. Almost simultaneously with the more complete report of the fat-deficiency syndrome by Burr and Burr *(11)*, McAmis, Anderson, and Mendel *(53)* likewise observed that rats receiving fats in their diets grew better than animals on control fat-free regimens; however, the latter workers failed to note the presence of the deficiency symptoms in the fat-free group. The ultimate proof of the fact that fat deficiency produces a deficiency disease should be credited to Burr and Burr *(11)*; these workers demonstrated that the symptoms which developed were not to be ascribed to the lack of the fat-soluble vitamins. These workers later showed *(12)* that the deficiency symptoms disappeared immediately when lard, cod-liver oil, or liver was fed. The curative agent was found to occur in the saponifiable fraction. The polyunsaturated acids, linoleic, linolenic, and arachidonic, were proved to be excellent curative agents while the saturated acids such as stearic, palmitic, myristic, and laurie, as well as the monoethenoid acid, oleic, were all entirely ineffective in relieving the fat-deficiency symptoms. Glycerol was likewise early shown to be without activity in this respect *(11)*.

In the early investigation of essential fatty acid deficiency, the failure in growth and the appearance of skin symptoms were the only positive effects to be identified. However, more recently, it has been shown that essential fatty acids play an essential role in making possible successful pregnancy and lactation; these dietary components likewise have been found to serve as protective agents against x-irradiation and in preventing the development of permeability of the skin capillaries. Finally, it has most recently been recorded that the essential fatty acids are important in assuring the normal distribution of cholesterol in the rat. The proof that essential fatty acids, obtainable by the animal only from dietary fat, are concerned in so many vital reactions as those listed above reinforces the idea of the essentiality of fats in the diet.

1. Growth. The classical demonstration of the necessity of essential fatty acids in the diet was based on their effect on growth. When weanling rats are placed on fat-free

diets, they grow normally for about 3 weeks; after that they continue to gain weight for 5 to 7 weeks but at a subnormal rate as compared with rats receiving cottonseed oil or other similar fats in the diet. Maximum body weights are usually attained in 10 to 12 weeks on fat-free diets, after which they remain fairly constant or start to decline *(21)*.

When linoleic, linolenic, or arachidonic acid or fats containing such acids are fed to the fat-depleted rats, the body weights of such animals increase, the skin lesions disappear, and the condition of the animals is restored to normal. The effectiveness of the essential fatty acids or of fats in alleviating the fat-deficiency syndrome is a function of the amount and type of essential fatty acids made available to the animal. On the basis of the demonstration by Greenberg *et al. (38)* that the curve for the increase in body weight plotted against the log dose of the essential fatty acid is a straight line, a method for assay of essential fatty acids has been devised. The rationale is based upon that developed for the bioassay of vitamin A by Coward *(17)*. Thomasson *(73)* has recently suggested a somewhat modified bioassay procedure which involves weight changes in animals with a restricted water intake.

Although it is entirely certain that an exact relation obtains between essential fatty acid intake and the extent of growth, it has not been proved whether or not the superior behavior of fat in providing growth of rats is to be entirely ascribed to its essential fatty acid content.

2. In the Metabolism of the Skin. The most pronounced symptom of fat-deficiency in all species is the eczematous condition of the skin. This is reflected by the scaliness of the tail and paws in the mouse, rat, and dog, and of the tail of the calf, together with roughness of the skin. The dermatitis which occurs is somewhat similar to that arising in pyridoxine (B_6) deficiency; in fact, Birch *(8)* has observed that the onset of the dermatitis obtained on vitamin B_6-deficient diets can be delayed by certain fats. It is believed that the unsaturated fatty acids do not replace pyridoxine but simply delay the onset of the skin symptoms *(61)*.

All of the three common essential fatty acids are effective in clearing up the skin symptoms resulting from fat deficiency. Linoleic acid is believed to be more effective than linolenic acid *(60, 63, 68)*. The ability of natural fats to bring about recovery from the skin disorders has been found to be proportional to their linoleate content *(10)*. According to Hansen *et al. (40)*, the absence of fat from the diet brings about definite alterations in both the epidermis and the dermis; moreover, the hair follicles, sebaceous glands, and capillaries are all affected. All changes were found to be reversible *(41)*.

3. In Protection from X-Irradiation. Although many different substances have been observed to modify the course of x-irradiation injury, fats have been found to have a specific effect in this condition. Decker *et al. (18)* were the first to report that a typical essential fatty acid deficiency could be produced in mice by x-irradiation. Cheng and her co-workers *(15)* observed that rats receiving diets containing 2, 15, or 30% of cottonseed oil derived marked protection from multiple sublethal doses of x-rays as compared with the situation noted in rats on a fat-free diet.

The protective effect of cottonseed oil against x-irradiation was proved to be a function of the essential fatty acid content. Thus, it was found that an increased survival could invariably be noted in fat-depleted rats subjected to 300 *r* weekly, when 10 mg. of ethyl linoleate was administered daily *(20)*. Subsequently, Cheng and associates *(16)* established that the optimum protective dose of linoleate against x-irradiation injury probably exceeds 100 mg. per day. The average survival time, and especially the periods at which LD_{25}, LD_{50}, and LD_{75} were reached, became progressively greater when the protective dose of linoleate tested was 10, 50, or 100 mg. of linoleate daily. The protective effect of fat against x-ray has been found to occur under a wide variety of conditions of exposure to x-rays both as related to the intensity of the dose and to the frequency of the exposure *(14)*.

The reason for the beneficial effect of essential fatty acids in x-ray injury is not entirely clear. It is possible that, since essential fatty acids are required for new tissue growth, animals are able to repair damage due to x-irradiation sufficiently promptly so that death may be avoided. In the absence of such prompt tissue regeneration, one might expect that death would supervene.

4. In Protection from Capillary Fragility. As early as 1939, Kramár and Kovács *(48)* made the first clinical observations that the resistance of the skin capillaries in

human subjects, especially in those with allergic manifestations, was higher on a dietary regimen containing a vegetable fat than on one composed entirely of fats of animal origin. Subsequently, Kramár and Levine *(49)* were able to prove that the capillary resistance in immature rats fed on a diet practically devoid of the fat and essential fatty acids, or on one containing 8% of methyl stearate, began to decrease within 7 weeks and reached pathologically low values shortly thereafter. The changes in capillary permeability could be entirely avoided if 5% of corn oil was added to the same basic diet; moreover, after this deficiency symptom had been established, it could be completely cured and the rats restored to normal by the administration of linseed oil or pure linoleate in therapeutic amounts.

The changes in capillary resistance were found to be inversely proportional to the extent of capillary permeability. That these symptoms represent another example of a function controlled by the essential fatty acids is demonstrated not only by their response to small amounts of essential fatty acids but also by the fact that they occur simultaneously with other recognized symptoms of fat deficiency such as the flattening of the weight curve.

5. In Pregnancy and Lactation. It has already been indicated that fats play an important role in successful pregnancy and lactation. There is now proof that at least a part if not all of the beneficial effects of this foodstuff are to be ascribed to its content of essential fatty acids.

Evans and co-workers *(27, 28)* were the first to point out that normal reproduction is impossible in the absence of the essential fatty acids. They noted that the mothers are unable to nurse their young on such fat-free diets, and that sterility develops in the male rats *(29)*. Small amounts of essential fatty acids were found to prevent the development of these symptoms as well as to cure these conditions when once established.

Several recent studies have given more information on the quantitative aspects of the essential fatty acid requirements. Quackenbush *et al. (59)* observed that the quantity of nutrients required for reproduction was about twice that necessary for the cure of the dermal lesions produced by a fat-free diet. Linolenic acid was recorded as being ineffective in allowing a normal reproduction while linoleic and arachidonic acids were recorded as having about the same biopotency in supplying the needs for pregnancy. More recently, Deuel *et al. (22)* have concluded that dietary fats are not required by the female rat for conception or for the completion of pregnancy when the diet is otherwise complete; however, in the absence of fat in the diet, the pups did not survive, presumably because of the mothers' inability to nurse their young. Although, in some cases, doses of cottonseed oil as low as 10 mg. were found to be sufficient to insure the survival of the pups for 3 days, as much as 200 mg. of this vegetable oil were required daily for optimum survival of the young over 21 days. Since comparable results were obtained with a daily feeding of 80 mg. of linoleate, it was concluded that the essential fatty acids constitute the active protective component of fats insofar as pregnancy is concerned.

Fat has long been known to stimulate lactation *(54-56)* although Loosli *et al. (51)* failed to prove that linoleate was the essential factor in the case of the rat; however, corn oil did produce a stimulatory effect while hydrogenated coconut oil was ineffective. In the tests of Deuel *et al. (22)*, the lowest mortality rate and the highest weaning weights of rats obtained when the nursing mothers received 100 or 200 mg. of cottonseed oil daily or 80 mg. of linoleate. It was concluded that a certain definite level of essential fatty acids is required for satisfactory lactation as well as for successful pregnancy.

6. In Cholesterol Metabolism. Fats have been widely maligned in recent years because of their reputed relationship to cholesterol. Since atheromas contain a considerable quantity of cholesterol, it is believed that any condition which augments the cholesterol supply may also aggravate the formation of atheromas and the attendant development of atherosclerosis.

Cholesterol is a natural product which is normally present in every cell of the body. In nervous tissue cholesterol makes up a large portion of the structural tissue. It is the parent substance of bile salts and of a number of steroid hormones, including the adrenocortical hormones and the male and female sex hormones. 7-Dehydrocholesterol also plays the role of vitamin D_3.

Some fats may serve directly as a source of cholesterol since they contain definite

amounts of this alcohol dissolved in them. Cholesterol is present only in animal fats; thus, butter has been reported to have as much as 280 mg. % *(58)*, egg-yolk fat a maximum of 2000 mg. % *(58)*, and lard as high a concentration as 120 mg. % *(44)*. Vegetable fats do not contain cholesterol but rather the related phytosterols. There is widespread evidence that the phytosterols cannot serve as a source of cholesterol in the animal body *(7, 69, 70)*.

In addition to certain fats being a source of preformed cholesterol, it is believed that both animal and vegetable fats may serve as building material for endogenous cholesterol by virtue of the fact that the two-carbon fragments of fatty acids can be built up into the sterol molecule. Although this deduction is a sound one, carbohydrates or proteins can likewise serve as a source of such acetate molecules, either directly or by way of the fat molecules which can be formed from either of these foodstuffs. For these reasons, the results of Alfin-Slater *et al. (5)*, which indicate that the level of newly-formed liver and plasma cholesterol in rats on a fat-low diet is unchanged when the animals are placed on a high-fat diet, are not surprising.

In addition to fats serving as sources of preformed cholesterol or of endogenously-formed cholesterol, the essential fatty acids function in an important manner in cholesterol transport and deposition. It has been recognized since the report of Kelsey and Longenecker *(45)* that the major portion of plasma linoleic acid is combined with cholesterol. Most recently it has been demonstrated that on a fat-free diet the level of liver cholesterol becomes elevated in rats concomitantly with a decrease in the level of plasma cholesterol *(4)*. On the other hand, when the diet contains 12.5% of cottonseed oil, the level of liver and adrenal cholesterol is at the normal low value while the plasma cholesterol is not reduced. The values of liver cholesterol were as follows in the several groups: control, 2.0 mg./gm.; 12.5% cottonseed oil diet, 2.06 mg./gm.; and fat-free diet, 4.48 mg./gm. The plasma cholesterol values in the three groups respectively were 65 mg. %, 64.4 mg. %, and 43.0 mg. %. It would thus appear that fat not only does not cause cholesterol deposition in the rat but that it facilitates its transport and its metabolism presumably through the mediation of the essential fatty acids. Kinsell and co-workers *(46, 47)* have reported that the blood cholesterol level of patients having high cholesterol values as a result of diabetes or related conditions can be lowered by the administration of vegetable fats but not by that of animal fats; it is possible that this effect in man may be associated with the effect of the essential fatty acid content of the fats.

CONCLUSIONS

Much evidence has accumulated to support the hypothesis that fats are essential rather than optional components of the diet. Whether or not the essentiality of fats is based entirely on their being sources of the so-called essential fatty acids is a moot question. However, the pronounced effect of fats on growth, on pregnancy, and lactation, on nitrogen-sparing action, on work capacity, and on period of survival indicates the profound nature of behavior of fat. The specific behavior of the essential fatty acids in the prevention of x-irradiation injury, and in preventing capillary permeability, as well as in regulating cholesterol deposition and metabolism, offers additional evidence of the importance of fats in the diet.

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THE EFFECT OF SORBIC, PROPIONIC, AND CAPROIC ACIDS ON THE GROWTH OF CERTAIN CLOSTRIDIA[a]

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Food preservatives, that are harmless as a component of human and animal diets, are added to foods for a variety of purposes. Some act as antioxidants to stabilize fat against oxidative deterioration, others act to maintain color quality, and others to prevent mold and yeast propagation. There is no reason to expect any one particular food preservative to function in all capacities. For example an antimycotic agent should not be expected to protect foods against all types of food poisoning bacteria. The important factor to be considered with regard to the growth of the food poisoning bacteria, is that the added food preservative, be it an antimycotic, antioxidant or of any other class, must not stimulate the growth of the food poisoning bacteria to make the food more likely to be responsible for toxic reactions. It is highly unlikely that any material will ever become available which can protect foods in general against all forms of microbiological spoilage without being toxic to the consumer of the foods. If an antimycotic material, harmless to the human or animal, exhibits an inhibitory effect on some of the food poisoning bacteria and is not stimulatory of the others, then there is additional reason to supplement foods with the antimycotic material. However, under no circumstances should there be tolerated a relaxation in sanitary techniques because a harmless food preservative has been added to a food.

Gooding *(4)* discovered that certain of the alpha, beta unsaturated fatty acids are good fungistatic agents for foods and food wrappers. Sorbic acid falls into this category. In many instances it has been shown that sorbic acid will replace sodium benzoate for the prevention of mold and yeast spoilage in various foods. In contrast to benzoate, Deuel and co-workers *(1)* found that sorbic acid is metabolized as are the other food fatty acids. Benzoate, however, must be detoxified in the liver and thence excreted. Deuel *et al. (2)* have also shown that concentrations of sorbic acid comparable to those of benzoate show no toxicity in rats and dogs. No harmful conditions are produced in dogs and rats with 5% sorbate.

Emard and Vaughn *(3)* have found that sorbic acid permitted the growth of lactic acid bacteria. This has proved to be a fortunate development since these organisms are added in the curing of cheese and pickles, foods which are susceptible to spoilage due to mold and yeast propagation. In the Emard-Vaughn paper, it was also reported that the *Clostridia* were not inhibited by sorbic acid in the concentrations employed. Unfortunately, the authors failed to present data on whether or not the *Clostridia* exhibited better or poorer growth in the sorbic acid supplemented medium than in the control medium containing no sorbic acid. With respect to

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other possible genera tested, encompassing bacterial species associated with food poisoning, Emard and Vaughn found that sorbic acid inhibited the species of *Salmonella* under all conditions studied. This would then represent an additional advantage when sorbic acid is used as an antimycotic agent in foods. Some species of *Streptococcus faecalis* were also inhibited by 0.12% sorbic acid *(3)*. The species of *Staphylococcus aureus* were inhibited by 0.07% sorbic acid in glucose-yeast extract media, while 0.12% sorbic acid was required in liver infusion media for complete inhibition; at lower concentrations of 0.08 to 0.10%, only some of the cultures were inhibited in the more favorable media *(3)*. In the case of *Staphylococcus aureus,* data were included in the paper *(3)* showing by turbidimetric measurements the degree of growth in both the control and in the sorbic-acid supplemented medium. At pH 5.1 growth occurred in the glucose broth controls and at pH 5.9 greater growth was noted, but not at all in the same media with the added sorbic acid. At pH 6.3 or 7.0 more growth was noted in the controls and some growth in the sorbic-acid supplemented media, but to a degree of one-third to one-half that of the controls. These findings merit further study since various species of the *Staphylococcus* genus are frequently involved in food poisonings. If those foods capable of fostering the growth of these microorganisms can be made resistant by adding sorbic aid and by lowering the pH to 6.0 or less, there would be still another reason for sorbic acid supplementation over and above antimycotic activity.

Further investigations by York and Vaughn *(8, 9)* have shown that many strains of *Clostridium botulinum* were tolerant to 3.0% sorbic acid. Unfortunately the authors again failed to state in their abstracts *(8, 9)* whether or not the growth observed was better or poorer than that noted in the unsupplemented control media. The fact that sorbic acid is utilized by the *Clostridia* is nothing more than a demonstration that sorbic acid like other nutrients in the media is metabolized by the microorganism. Deuel and co-workers *(1)* have shown that sorbic acid is as readily metabolized as the naturally-occurring caproic acid by the animal organism. Melnick and associates *(6)* have demonstrated that molds can also metabolize sorbic acid even during the period of growth inhibition.

Since the York and Vaughn abstracts *(8, 9)* were incomplete, studies were initiated in our laboratory to determine whether or not sorbic acid is a growth stimulant for the *Clostridia.* In addition, the influence of the naturally-occurring caproic acid (saturated sorbic acid) and of propionic acid were similarly evaluated. The latter is a well-known food preservative also used for its antimycotic activity.

EXPERIMENTAL

Three bacterial cultures were used in this work, *Clostridium sporogenes* ATCC 8074, *Clostridium botulinum* NCA 109 Ac and *Clostridium botulinum* NCA 1267 Be. The three acids investigated were sorbic, propionic, and caproic. The organisms studied were catalase negative.

The liver infusion was prepared as recommended in the ninth edition of Difco Manual. The final broth was prepared according to McClung and McCoy *(5)* and Vaughn *(7)*. The glucose-yeast extract broth was prepared according to Emard and Vaughn *(3)*.

To each tube of broth, containing the requisite percentages of the acids under investigation, was added one drop of a 48 hr. liver broth culture of the respective

organisms. Total volume in all tubes was 10 ml. All tubes were overlayed with a 50-50 paraffin-vaseline seal and incubated at 37°C. Readings were made by visual turbidity, independently by each of the authors, after 1, 2, and 4 days of incubation, with agreement obtained in evaluating degrees of growth.

RESULTS

The results in Table 1 show that sorbic, propionic, and caproic acids are neither inhibitory nor stimulatory to *Clostridium sporogenes* and *Clostridium botulinum*, types A and B, in the absence of phosphate.

The results in Table 2 show no stimulation of the same organisms, and no bacteriostatic effect by the fourth day when the phosphate is present. Early in the incubation period there seems to be some inhibition of the anaerobes.

TABLE 1

Growth of Clostridia on liver infusion broth alone or supplemented with caproic, propionic, or sorbic acid

Unbuffered **pH = 6.7**

Supplement added to liver infusion broth	Cl. sporogenes[1]		Cl. botulinum A[1]		Cl. botulinum B[1]	
	2nd day[2]	4th day	2nd day[2]	4th day	2nd day[2]	4th day
No supplement (control)	4+	4+	4+	4+	3+	4+
Caproic acid (0.12%)	4+	3+	3+	4+	3+	4+
Propionic acid (0.12%)	4+	3+	4+	4+	4+	4+
Sorbic acid (0.12%)	4+	4+	4+	4+	2+	4+

[1] Cultures obtained originally from National Canners.
[2] No growth on first day.

TABLE 2

Growth of Clostridia on liver infusion broth alone or supplemented with caproic, propionic, or sorbic acid

Phosphate buffer (0.1%) **pH = 6.7**

Supplement added to liver infusion broth	Cl. sporogenes[1]		Cl. botulinum A[1]		Cl. botulinum B[1]	
	2nd day[2]	4th day	2nd day[2]	4th day	2nd day[2]	4th day
No supplement (control)	3+	4+	4+	4+	4+	4+
Caproic acid (1%)	3+	3+	—	3+	1+[3]	4+
Propionic acid (1%)	1+	1+	—	[illegible]	2+	4+
Sorbic acid (1%)	3+	3+	2+	[illegible]	3+	4+

[1] Culture obtained originally from National Canners.
[2] No growth on first day.
[3] No gas formation.

TABLE 3

Growth of Clostridia on glucose yeast extract broth alone or supplemented with caproic, propionic, or sorbic acid

Unbuffered **pH = 6.7**

Supplement added to glucose-yeast broth	Cl. sporogenes[1]		Cl. botulinum A[1]		Cl. botulinum B[1]	
	1st day	4th day	1st day	4th day	1st day	4th day
No supplement (control)	4+	4+	4+	3+	4+	4+
Caproic acid (0.12%)	4+	2+	1+[2]	1+	4+	3+
Propionic acid (0.12%)	4+	4+	4+	4+	4+	4+
Sorbic acid (0.12%)	4+	3+	4+	3+	4+	4+

[1] Culture obtained originally from National Canners.
[2] No gas formation.

TABLE 4

Growth of Clostridia on glucose yeast extract broth alone or supplemented with caproic, propionic, or sorbic acid

Phosphate buffer (0.1%) **pH = 6.7**

Supplement added to glucose-yeast broth	Cl. sporogenes[1]		Cl. botulinum A[1]		Cl. botulinum B[1]	
	1st day	4th day	1st day	4th day	1st day	4th day
No supplement	4+	4+	4+	3+	4+	4+
Caproic acid (1%)	1+[2]	2+	—	1+	—	3+
Propionic acid (1%)	3+	3+	1+[2]	4+	3+	4+
Sorbic acid (1%)	2+[2]	2+	—	3+	1+[2]	4+

[1] Culture obtained originally from National Canners.
[2] No gas formation.

The same results are obtained with glucose-yeast extract broth with and without phosphate (see Tables 3 and 4). The glucose-yeast extract broth was found to be better than the liver infusion broth for the growth of these anaerobes, due probably to the presence of a readily available carbon source.

The concentrations of sorbic acid, propionic acid, and caproic acid used were 0.12% in the case of the unbuffered medium and 1.0% in the medium containing the phosphate buffer. Since higher concentrations of sorbic acid are not intended as food supplements, information on concentrations above 1.0% would merely be of academic interest.

Shortly after the completion of the present studies, the authors heard the York-Vaughn paper *(9)* presented orally on June 29, 1954, at the Fourteenth Annual Meeting of the Institute of Food Technologists. Data were presented to show that, whereas the *Clostridia* were tolerant of sorbic acid, growth was not stimulated when sorbic acid was added to the media. Thus, the present findings in no way contradict those obtained by York and Vaughn *(8, 9)*; indeed our findings are regarded to be merely of confirmatory value. It is anticipated that the full report by York and Vaughn will include sufficient data to show, as we have found, that sorbic acid does not inhibit or stimulate the *Clostridia*.

SUMMARY

Sorbic acid, propionic acid, and caproic acid are similar in that they do not inhibit or stimulate the growth of *Clostridium botulinum*, types A and B, and *Clostridium sporogenes*. Sorbic acid is recommended for antimycotic activity but not to prevent the growth of catalase negative organisms.

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DIFCO

Bacto-Dehydrated Media for Microbiological Assay of VITAMINS and AMINO ACIDS

These media contain all the necessary nutriments for the growth of specified test organisms for the microbiological assay of vitamins and amino acids except for the component under assay. These basal media require only the addition of specified increasing amounts of the vitamin or amino acid being assayed to obtain a growth response which may be measured by acidimetric or turbidimetric methods for the construction of standard curves. The vitamin or amino acid content of the material under assay is determined by adding appropriate concentrations of the test substance to the basal medium and comparing the growth response obtained with that of the standard.

BACTO-RIBOFLAVIN ASSAY MEDIUM
BACTO-NIACIN ASSAY MEDIUM
BACTO-THIAMIN ASSAY MEDIUM
BACTO-PANTOTHENATE ASSAY MEDIUM
BACTO-B_{12} ASSAY MEDIUM USP
BACTO-CS VITAMIN B_{12} AGAR
BACTO-FOLIC ACID ASSAY MEDIUM
BACTO-PYRIDOXINE ASSAY MEDIUM
BACTO-BIOTIN ASSAY MEDIUM
BACTO-CHOLINE ASSAY MEDIUM
BACTO-CF ASSAY MEDIUM
BACTO-TRYPTOPHANE ASSAY MEDIUM
BACTO-LEUCINE ASSAY MEDIUM
BACTO-METHIONINE ASSAY MEDIUM
BACTO-LYSINE ASSAY MEDIUM
BACTO-ISOLEUCINE ASSAY MEDIUM
BACTO-ARGININE ASSAY MEDIUM
BACTO-TYROSINE ASSAY MEDIUM

The method employed in carrying stock cultures of the test organisms and preparing the inoculum for microbiological assay is important. The following media have been developed especially for carrying stock cultures and for preparation of the inoculum:

BACTO-MICRO ASSAY CULTURE AGAR
BACTO-MICRO INOCULUM BROTH
BACTO-NEUROSPORA CULTURE AGAR

BACTO-VITAMIN FREE CASAMINO ACIDS, dehydrated, is an acid hydrolysate of vitamin free casein prepared especially for laboratories investigating microbiological assay of vitamins.

Descriptive literature available upon request

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